

# **Investigating structure-function relationships of dough stickiness within low sodium bread dough formulations**

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## ABSTRACT

The overarching goal of this research was to gain a greater understanding of the underlying structure-function relationships leading to the sticky dough phenomenon when sodium chloride is reduced in bread dough formulations. The primary objectives of this research were to: a) examine the effect of flour composition (focusing on gluten content/quality) on the dough handling properties of four flours ranging from good to poor dough handling; b) examine the effect of NaCl level on the dough handling properties, morphology (focusing on the gluten network formation), stickiness and water mobility with the same four flours; and c) examine the effect of various salts from the lyotropic series on the dough handling properties, morphology, stickiness and water mobility of a known strong/non-sticky dough producing flour and a weak/sticky dough producing flour to achieve similar properties to that of NaCl.

Within Chapter 3, the chemical compositions of flours milled from four different Canada Western Red Spring (CWRS) wheat cultivars (i.e., Pembina, Roblin, McKenzie and Harvest) were investigated and then related to the rheological properties, stickiness, morphology and water mobility of each dough with 2% NaCl. All cultivars showed similar proximate composition, with the exception of the protein content, and were all of high quality with minimal enzymatic activity/degradation. Major differences were noted for flour cultivars with respects to gluten quality and damaged starch level. Pembina and Roblin, which are both known strong dough producing flours, showed a significantly higher gluten index and gluten performance index than both McKenzie and Harvest, which are both known to be intermediate and weak dough producing flours, respectively. However, dough prepared with Pembina was found to have the greatest resistance to extension relative to the other flour cultivars. Pembina was found to have greater amounts of low molecular weight glutenin subunits (insoluble) than Roblin which could account for Pembina's greater resistance to extension than Roblin. McKenzie and Harvest flours had higher levels of gliadin than Pembina and Roblin, in part accounting for the weaker doughs. McKenzie and Harvest both had significantly higher damaged starch (~7.1%) (i.e., harder kernels) than Pembina and Roblin (~5.7%) which would impact the hydration of gluten proteins and therefore would ultimately impact the formation of the gluten network.

Within Chapter 4, the dough rheological properties, stickiness, morphology and water mobility for the four CWRS wheat cultivars were examined as a function of NaCl (0-4%). More

specifically the dough rheology was investigated with respect to the oscillatory shear, creep recovery and extensibility. The loss tangent of doughs prepared with Roblin, McKenzie and Harvest flours had similar values and greater than that of dough prepared with Pembina flour. This trend was similar to the strength trend seen in Chapter 3 with the examination of resistance to extension. Rheological data indicated that with increasing NaCl levels doughs prepared with the four cultivars increased in strength. The magnitude of changes in dough strength with different NaCl levels tended to be cultivar specific. For dough stickiness Pembina and Roblin showed the least stickiness when compared to McKenzie and Harvest at the 0 and 2% NaCl levels, with the addition of 2% NaCl decreasing stickiness for all cultivars. However at the 4% NaCl level a greater cultivar effect was observed with regard to stickiness. Water association measurements (i.e., distribution of water as free, associated with starch or associated with gluten) found that with the addition of NaCl there was a decrease in free water among the doughs prepared with the different cultivars and an increase in the water associated with the starch-fraction. Overall, Pembina and Roblin formed stronger gluten networks with lower stickiness than McKenzie and Harvest and NaCl sensitivity was found to be cultivar dependent. Pembina was chosen as a strong/non-sticky dough producing flour and Harvest was chosen as a weak/sticky dough producing flour to move forward to Chapter 5 to investigate the effect of salts from the lyotropic series.

Within Chapter 5, the impact of salts from the lyotropic series ( $\text{NH}_4\text{Cl}$ ,  $\text{KCl}$ ,  $\text{NaCl}$ ,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ , and  $\text{MgSO}_4$ ) at the 1 and 2% salt levels on the dough rheology, morphology, stickiness and water mobility of doughs prepared using a CWRS flour producing a strong/non-sticky dough (Pembina) and a flour producing a weak/sticky dough (Harvest), were investigated. Overall, Pembina developed stronger gluten networks than Harvest as determined by a lower loss tangent and reduced amount of deformation during creep recovery. However, the effect of salt-type was dependent on the cultivar. For instance, in the case of Pembina only dough prepared with  $\text{NH}_4\text{Cl}$  was found to experience significantly reduced deformation during creep recovery compared to  $\text{NaCl}$ , whereas all other salt-types were similar. However for Harvest,  $\text{KCl}$ ,  $\text{CaCl}_2$  and  $\text{MgCl}_2$  were found to have a weakening effect on the gluten network with respect to the higher deformation experienced when compared to  $\text{NaCl}$ ; whereas  $\text{NH}_4\text{Cl}$  and  $\text{MgSO}_4$  resulted in lower deformation compared to  $\text{NaCl}$ . Overall Pembina had lower dough stickiness in all cases when compared to Harvest. Dough stickiness saw the greatest decrease for both flour cultivars

with the use of  $\text{NH}_4\text{Cl}$ . Enhanced dough morphology was noticed for Pembina and Harvest in the presence of  $\text{NH}_4\text{Cl}$ . Findings from the rheology and stickiness measurements indicate  $\text{NH}_4\text{Cl}$  could serve as a replacement for  $\text{NaCl}$  in low sodium dough formulations, however future studies are necessary to determine the impact on final loaf quality, consumer acceptability and potential health implications.

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## LIST OF SYMBOLS AND ABBREVIATIONS

AA	Amino acid
ANOVA	Analysis of variance
BA	Baking absorption
BU	Barbender units
BWBD	Bandwidth breakdown 1 min after peak
BWPR	Bandwidth at peak dough resistance
CLSM	Confocal laser scanning microscopy
CWRS	Canada Western Red Spring
Da	Dalton
d.b.	Dry basis
DDT	Dough development time
DSC	Differential scanning calorimetry
DTG	Derivative thermogravimetric curve
FAB	Farinograph water absorption
FD	Fractal dimension
FWC	Freezable water content
Gli	Gliadin
Glu	Glutenin
GPI	Gluten performance index
G'	Storage modulus (elastic modulus)
G''	Loss modulus (viscous modulus)
G*	Complex modulus
HAR	Harvest
HI	Hardness index
HMW-GS	High molecular weight glutenin subunit
J <sub>el</sub>	Relative elasticity
J <sub>max</sub>	Maximum creep compliance
LMW	Low molecular weight
LMW-GS	Low molecular weight glutenin subunit

MC	McKenzie
MDT	Mixograph development time
MTI	Mixing tolerance index
MW	Molecular weight
NSP	Non-starch polysaccharide
PDR	Peak dough resistance
PEM	Pembina
RBD	Resistance breakdown 1 min after peak
ROB	Roblin
RVU	Rapid viscoanalyzer units
R/E	Resistance to extension/extensibility
SKCS	Single kernel characterization system
SRC	Solvent retention capacity
STA	Stability time
$\tan \delta$	Loss tangent
TGA	Thermogravimetric analysis
Tq.	Torque
w.b.	Wet basis
WIP	Work input to PDR
$\alpha$	Alpha
$\gamma$	Gamma
$\omega$	Omega

# **1. INTRODUCTION**

## **1.1 Overview**

Society's consumption of NaCl is far too high; almost double the recommended intake levels per day. The majority (~77%) of that salt intake comes from processed foods (Mattes & Donnelly, 1991; Health Canada, 2012). This over consumption of sodium has been linked to the presence of hypertension, which is a major contributing factor in cardiovascular disease which, in turn, accounts for a major portion of the total global deaths (Miller & Hoseney, 2008; Lynch et al., 2009; Belz et al., 2012). This is a preventable strain that is put on the health care system. Therefore, to combat high sodium consumption Health Canada created the sodium reduction strategy, recommending that the food industry decrease the use of sodium in formulations to lower consumer consumption to ultimately reduce the number of hypertension cases (Health Canada, 2012). Many processed foods can reduce the sodium content without loss of product functionality; however flavour loss may occur. Whereas for some food products, such as bread, sodium chloride is necessary for product structure and functionality (Farahnaky & Hill, 2007; Miller & Hoseney, 2008; Lynch et al., 2009; Belz et al., 2012).

Because bread is commonly consumed within many households, Health Canada has recommended that the sodium content in 100 g serving of bread be brought down to 330 mg from 470 mg (Health Canada, 2012). This has proven to be a major challenge within industrial bread production facilities because the reduction of sodium within dough formulations compromises dough rheology and handling due to the occurrence of a sticky dough phenomenon, resulting in poor final product quality and costly production shutdowns (Adhikari et al., 2001; Farahnaky & Hill, 2007; Belz et al., 2012; Israr et al., 2016). It is well known that sodium chloride is one of the key ingredients in producing dough suitable for bread production because it impacts the development of the gluten network during the mixing stage by increasing the time necessary for protein/starch hydration to achieve optimal dough rheological properties and a non-sticky dough (Uthayakumaran, 2011; Belz et al., 2012). Stickiness is a property that is related to the adhesive forces (i.e., between the dough and the mixing surface) and the cohesive forces (i.e.,

protein-protein interactions creating the gluten network) (Adhikari et al., 2001; van Velzen et al., 2003) and is suggested to be dependent on the viscoelastic properties of the dough (Dobraszczyk, 1997; Hoseney & Smewing, 1999). Therefore, measurements of the rheological properties of the dough give an indication of the cohesive properties within the dough (Dobraszczyk, 1997; Hoseney & Smewing, 1999). Dough stickiness occurs when the adhesive forces are higher than the cohesive forces within the dough (Hoseney & Smewing, 1999). It has been suggested that the occurrence of dough stickiness is impacted by processing parameters such as mixing conditions (e.g., temperature, time, shear), level of flour component hydration and formulation (e.g., flour, salt, etc.) as all of these can impact the viscoelastic properties of the dough (Dhaliwal et al., 1990; Dobraszczyk, 1997; van Velzen et al., 2003; Miller & Hoseney, 2008; Beck et al., 2012a).

Sodium chloride serves to reduce dough stickiness by impacting the rheological properties of the dough through the development of the gluten network during the mixing stage (Farahnaky & Hill; 2007; Beck et al., 2012a; Belz et al., 2012). Many reviews have stated that salt results in increased dough stability, mixing time, resistance to extension and extensibility, and elasticity; all of which are indications of a strong gluten network development (Miller & Hoseney, 2008; Belz et al., 2012; Israr et al., 2016). It is the gluten network that imparts the viscoelastic properties to the dough through the two gluten proteins, glutenin and gliadin. The gliadins contribute to the viscous component of the dough and the extensibility and are distributed throughout the backbone glutenin polymers; whereas the glutenins impart cohesiveness, elasticity and strength to the dough through the creation of an interconnected network (Wieser, 2007). The interactions that are important in the formation of the gluten network are non-covalent (hydrogen bonding, hydrophobic, and ionic) and covalent disulphide bonding that creates interconnections between the glutenin subunits (Wieser, 2007). During mixing these interactions can be manipulated with the use of NaCl. The salt shields the charges on the gluten proteins' surface, allowing the protein polymers to come into close contact through hydrophobic interactions, slowing the hydration of the gluten protein polymers, and allowing the proteins to interact (i.e., through hydrogen bonds and disulphide bonds) which forms a stronger gluten network (Preston, 1989; Butow et al., 2002; Miller & Hoseney, 2008; Uthayakumaran, 2011). By changing these interactions the formation of the gluten network is altered and with it the strength of the dough and potentially dough stickiness.

The role of NaCl and other alternative salts in shaping the nature of interactions within the dough matrix can be explored by studying the effects of salts from the lyotropic series (also known as the Hofmeister series) on the dough rheology and handling properties (Salovaara, 1982a; Preston, 1989; Miller & Hoseney, 2008). Both anions and cations are ranked in order of the most stabilizing to destabilizing effects on protein-water interactions (He et al., 1992; Miller & Hoseney, 2008). Stabilizing ions lead to less protein hydration, more structure and decreased protein solubility (i.e., ion-water interactions are favoured, leading to increased protein-protein interactions), whereas destabilizing ions lead to greater hydration and increased protein solubility (i.e., protein-water interactions are favoured, leading to weaker protein-protein interactions). As a result, depending on the anions or cations present within the formulation, the level of hydrophobic interactions and hydrogen bonding occurring in the system can be altered (Preston, 1989; Butow et al., 2002; Miller & Hoseney, 2008).

## **1.2 Objectives**

The overall goal of this research was to gain a greater understanding of the underlying mechanisms leading to the sticky dough phenomenon within a low sodium environment. The effect of NaCl levels on the nature of interactions will be examined using flours known for their weak and strong gluten network producing properties. For instance, Roblin and Pembina have been observed to have good dough handling properties (i.e., non-sticky dough) both at high (~470 mg/100 g bread) and low (~330 mg/100 g bread) NaCl levels, whereas both McKenzie and Harvest have good and poor dough handling properties at high and low NaCl levels, respectively. The composition of these flours will be characterized as it relates to their protein and starch components. Various types of salts from the lyotropic series will be used to modify interactions within the gluten network prepared from the different cultivars, where dough rheology, dough stickiness, dough morphology and water mobility measurements will be used as indicators of formulation changes. The specific objectives are as follows:

- (a) To investigate the chemical composition of flours prepared from different Canada Western Red Spring wheat cultivars (Pembina, Roblin, McKenzie and Harvest) which display good, average and poor dough handling properties within a low sodium environment.

- (b) To investigate the effect of NaCl level on the morphology, dough handling properties and water mobility of dough prepared from different Canada Western Red Spring wheat cultivars as it relates to the formation of the gluten network.
- (c) To investigate the effect of different salt types from the lyotropic series on hydrophobic and hydrogen bonding of gliadins and glutenins with both sticky and non-sticky dough as it relates to water mobility, dough rheology, dough stickiness, and dough morphology.

### **1.3 Hypotheses**

To achieve the overall goal of this research, the following hypotheses will be tested:

- (a) Flours containing higher quality gluten (i.e., higher amounts of glutenin than gliadin, more specifically, higher amounts of the high molecular weight glutenin subunit [HMW-GS] fractions) will form stronger gluten networks resulting in less sticky dough. Dough stickiness will increase as gluten protein quality decreases due to decreased protein-protein interactions.
- (b) At higher NaCl levels there will be greater protein-protein interactions facilitated through hydrophobic and hydrogen bonding and thus a more viscoelastic and non-sticky dough. At lower NaCl levels there will be an increase in both water mobility and gluten network hydration resulting in an increase in dough stickiness.
- (c) Water mobility will be enhanced in the presence of chaotropic cations given that chaotropic ions weaken the gluten network and will create sticky dough, whereas the non-chaotropic ions will strengthen the gluten network and decrease water mobility, creating non-sticky dough.



## **2. LITERATURE REVIEW<sup>1</sup>**

### **2.1 ABSTRACT**

Large consumptions of dietary sodium have been shown to lead to hypertension, one of the main causative factors in cardiovascular disease. Bread (and other cereal products) account for ~30% of overall sodium intake in our diet, therefore industry has been developing strategies to significantly reduce its usage. However at reduced sodium levels, dough handling can be affected due to a sticky dough phenomena creating major processing issues and a poor quality final product. It is hypothesized that the formation of a strong gluten network plays a crucial role in developing non-sticky dough, a process which is strengthened in the presence of NaCl. However at low NaCl levels, a weaker gluten network forms resulting in the prevalence of other wheat flours' constituents impact on water mobility within the dough to contribute to the stickiness phenomenon. This review discusses the underlying mechanisms that can influence the formation of sticky dough within a low sodium environment, and discusses strategies used to help circumvent them.

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<sup>1</sup> Partially reproduced with permission. Avramenko, N. A., Tyler, R. T., Scanlon, M. G., Hucl, P., & Nickerson, M. T. (2016). The chemistry of bread making: the role of salt to ensure optimal functionality of its constituents. *Food Reviews International*, DOI: 10.1080/87559129.2016.1261296.

## 2.2 INTRODUCTION

High dietary sodium intake from processed foods represents a major health concern for consumers worldwide. The consumption of large amounts of sodium has been shown to lead to hypertension, which is one of the main causative factors in cardiovascular disease, accounting for one third of total global deaths (Miller & Hoseney, 2008; Lynch et al., 2009). Currently Canadians ingest ~3,400 mg of sodium per day, which is more than double the amount recommended (1,500 mg per day) by Health Canada (Health Canada, 2012). As a result, Health Canada developed a sodium reduction strategy that aims to achieve an average daily intake of sodium ~2,300 mg per day by 2016 by mandating lower sodium levels across a range of foods, including bread. Health Canada estimated that a decrease of 1,840 mg of sodium per day would result in a 30% reduction in cases of hypertension and would result in a direct annual cost savings of \$430 million for the health care system through fewer physician visits, laboratory tests and prescriptions (Joffres et al., 2007; Health Canada, 2012). Processed foods, such as soups, meat/fish, bakery products, breakfast cereals and dairy products/alternatives account for ~77% of total sodium intake (Mattes & Donnelly, 1991).

Bread (along with other cereal-based products) is one of the most widely consumed products in the human diet, and as such accounts for ~30% of overall sodium intake (Farahnaky & Hill, 2007; Miller & Hoseney, 2008; Lynch et al., 2009). In 2009-10, the sodium content in 100 g of bread was ~470 mg. Health Canada's sodium reduction strategy aims to reduce sodium levels in three phases, from 470 mg per 100 g bread to 430 mg, then to 380 mg, and then finally to 330 mg by the end of 2016 (Health Canada, 2012). Currently the bread industry has been effective at reducing the sodium content in white bread down to 380 mg per 100 g bread through re-formulations and the use of sodium alternatives such as potassium chloride. However the latter can result in unacceptable bitter/metallic tastes (Salovaara, 1982a; Miller & Hoseney, 2008; Belz et al., 2012). Upon reducing sodium levels, dough rheology and handling can be compromised due to a sticky dough phenomenon causing major processing issues and a poor quality final product (Farahnaky & Hill, 2007). Dough stickiness occurs when there are high adhesive forces (interactions between the dough and mixing surfaces) and low cohesive forces (interactions within the dough) (Adhikari et al., 2001; van Velzen et al., 2003). Sticky dough results in low dough mixing tolerance and reduced dough strength and, if in excess, costly disruptions in production due to adherence of dough to the processing equipment (Dobraszczyk,

1997; Adhikari et al., 2001; van Velzen et al., 2003). The presence of sticky dough is affected by processing parameters such as the level of hydration, mixing conditions (e.g., temperature, time, shear) and formulation (e.g., flour, salt, etc.) (Miller & Hoseney, 2008; Beck et al., 2012a).

Sodium chloride is necessary for strengthening the gluten network and enhancing dough stability. NaCl serves to stabilize yeast fermentation, enhance product flavour, strengthen the gluten network and increase the dough mixing time for increased protein-protein interactions (Miller & Hoseney, 2008). Gluten within the wheat flour imparts dough properties such as extensibility, viscosity, elasticity, cohesiveness and contributes to water absorption, the extent of which is highly dependent upon the quality and ratio of the gliadin and glutenin proteins comprising the gluten matrix (Joye et al., 2009). Gliadins are proteins contributing to the extensibility of the gluten network (Joye et al., 2009), whereas glutenins are proteins that contribute to the elasticity and cohesiveness of the gluten network (He et al., 1992; Joye et al., 2009). In a flour-water system (pH ~6.0) the gluten proteins are below their isoelectric point (pH 7.5), giving them an overall net positive charge (Gennadios et al., 1993; Miller & Hoseney, 2008). Under ‘charged’ conditions, proteins repel one another and become more hydrated resulting in shorter mixing times because interactions between the proteins are less; creating weaker and stickier dough (Miller & Hoseney, 2008). Once NaCl is added, charged sites on the protein’s surface become shielded allowing proteins to interact and aggregate through hydrophobic interactions. Consequently, hydration of the gluten proteins is less and the dough formed is stronger (Preston, 1989; Butow et al., 2002; Miller & Hoseney, 2008).

## **2.3 DOUGH FORMULATION**

Dough is a complex, non-linear and time-dependent viscoelastic system comprised of a multitude of ingredients and phases (liquid, solids and gases) (Scanlon & Zghal, 2001; Jekle & Becker, 2011). Ingredients that are considered essential to the wheat breadmaking process are flour, water, salt and yeast, whereas nonessential ingredients may include fat, sugar, dairy products, enzymes, yeast food, emulsifying agents and improvers (Hoseney, 1998e; Collando-Fernandez, 2003; Moore, 2004; Goesaert et al., 2005; Lai & Lin, 2006). The latter are incorporated to enhance the dough-bread machinability, palatability and shelf life. Dough viscoelasticity arises from quality attributes of the flour, the level of water absorption/hydration, amount of entrapped gases, and mixing/kneading conditions (Salvador et al., 2006). A summary

of the functional role of both essential and nonessential ingredients is given in Table 2.1; however flour, water, salt and yeast will be discussed in greater depth.

### ***2.3.1 Role of wheat flour and its composition***

Wheat flour is the major ingredient in dough, and is unique relative to other cereal flours (barley, oat, rice and corn) since it forms strong viscoelastic networks upon hydration with water (Lai & Lin, 2006). For good breadmaking purposes, flours typically come from hard spring wheats such as the Canada Western Red Spring wheat (CWRS) class of cultivars because of the medium to strong dough handling properties of their flours. Hard wheat classes tend to have higher protein contents (~10-14%), making them more applicable for pan breads, whereas soft wheat classes have lower protein levels (~8-10%) making them unsuitable for breadmaking (Hoseney, 1998e; Lai & Lin, 2006; Delcour et al., 2012). The quality of flour and composition can vary with cultivar, the environment, agricultural (e.g., fertilizing, harvesting, etc.) and milling practices (Collando-Fernandez, 2003). Starch represents the major component in wheat flour (~70-75%) followed by water (~14%), protein (~10-12%), non-starch polysaccharides (NSP) (e.g., arabinoxylans) (~2-3%) and lipids (~2%) (Goesaert et al., 2005).

#### *Starch*

Amylopectin and amylose are two distinct polysaccharide molecules which make up starch, differing in both shape and size (Oates, 2001). Amylopectin (69-73%) is highly branched and large in size ( $10^7$  to  $10^9$  Da), whereas amylose (27-31%) has minimal branching and is smaller ( $10^5$  to  $10^6$  Da) in nature (Oates, 2001). Native wheat starch granules are water-insoluble with a bimodal size distribution, meaning that there are both small spherical granules (average diameter of ~5  $\mu\text{m}$ ) and large granules that are lenticular in shape (i.e., having the shape of a double convex lens) (average diameter ~20  $\mu\text{m}$ ) (Oates, 2001; Goesaert et al., 2005). Starch granules are inert entities and are structurally stable with a semi-crystalline structure making them birefringent (Oates, 2001; Goesaert et al., 2005). Starch is structurally complex with several levels of organization, where precise modeling of its complete structure is still under investigation. Damage can occur during the milling process resulting in a loss of birefringence to the damaged starch, increased water absorption and greater susceptibility to enzymatic

Table 2.1 Summary of the functional role of essential and nonessential ingredients within dough.

Ingredient	Functional role
<b>Flour</b> (Wheat)	<p>Structure</p> <ul style="list-style-type: none"> <li>- Major ingredient consisting mainly of starch, water and protein which imparts structure and body <sup>(1-5)</sup>.</li> <li>- Minor components important for quality are non-starch polysaccharides and lipids <sup>(2)</sup>.</li> <li>- Gluten proteins are crucial for structure formation <sup>(1-3)</sup>.</li> <li>- Starch is important for heat induced crumb formation <sup>(1,2)</sup>.</li> </ul>
<b>Water</b>	<p>Hydration</p> <ul style="list-style-type: none"> <li>- Hydrates the gluten proteins, starch and the non-starch polysaccharides and damaged starch and allows for the formation of a viscoelastic dough <sup>(3,5)</sup>.</li> <li>- Is a solvent for the other ingredients, medium for chemical and biochemical reactions, and aids in dough mobility <sup>(3,5)</sup>.</li> <li>- Has an effect on bread shelf life <sup>(3)</sup>.</li> </ul>
<b>Yeast</b> ( <i>Saccharomyces cerevisiae</i> , fresh or dried) <sup>(5)</sup> .	<p>Leavening</p> <ul style="list-style-type: none"> <li>- Converts simple sugars into CO<sub>2</sub> and ethanol, the fermentation products also impart flavour <sup>(1,3,4,5)</sup>.</li> <li>- Factors controlling rate of fermentation: temperature, nutrient supply, water content, pH, sugar content, salt content and type of yeast <sup>(5)</sup>.</li> </ul>
<b>Salt</b> (NaCl)	<p>Flavour/structure</p> <ul style="list-style-type: none"> <li>- Controls fermentation by inhibiting yeast activity to control bread expansion <sup>(1,3,4,5)</sup>.</li> <li>- Inhibits the hydration of gluten, thus strengthening the gluten network <sup>(1,3,4,5)</sup>.</li> <li>- Prolongs shelf life and imparts flavour <sup>(1,3,5)</sup>.</li> </ul>
<b>Shortening/fat</b> (soft fats such as hydrogenated vegetable fats or surface active materials like mono/diglycerides or lecithin) <sup>(1)</sup> .	<p>Lubricant/softener</p> <ul style="list-style-type: none"> <li>- Increases the shelf life, produces a finer grain, makes crust more elastic and softer through the formation of a film between the starch and protein layers <sup>(1,3,5)</sup>.</li> <li>- Creates easier slicing <sup>(5)</sup>.</li> <li>- Increases dough plasticity, resulting in less water necessary in the formulation <sup>(1)</sup>.</li> </ul>

<b>Sugars</b> (sugar (sucrose) or invert sugar: liquid sucrose broken down to monomers fructose and glucose) <sup>(5)</sup> .	Energy source for yeast <ul style="list-style-type: none"> <li>- Promotes fermentation, browning of the crust and imparts flavour <sup>(1,3,4,5)</sup>.</li> <li>- Tenderizes bread <sup>(1,3)</sup>.</li> </ul>
<b>Dairy products</b> (skim milk powder & whey containing lactose protein concentrate) <sup>(1,5)</sup> .	Nutrition/color <ul style="list-style-type: none"> <li>- Enhances nutritional profile of bread (high in lysine and calcium), imparts flavour, contributes to crust colour and softness, and provides buffering for dough <sup>(1,3,5)</sup>.</li> </ul>
<b>Enzymes</b> ( $\alpha$ -amylase, protease, glucose oxidase, xylanase) <sup>(1,3)</sup> .	Bread quality <ul style="list-style-type: none"> <li>- Amylase serves to convert starches into fermentable sugars as well as extend the shelf life of bread <sup>(3)</sup>.</li> <li>- Protease acts on the protein to decrease dough mixing time <sup>(3)</sup>.</li> <li>- Glucose oxidase and xylanase function to strengthen the dough <sup>(3)</sup>.</li> <li>- Fungal amylase and xylanase increase baked loaf volume <sup>(3)</sup>.</li> </ul>
<b>Yeast food</b> (either mineral yeast food or fermentable sugars: malted flour, malt extract) <sup>(1)</sup> .	Controls fermentation (mineral yeast food) <ul style="list-style-type: none"> <li>- Through the use of water conditioners (calcium salts), yeast conditioners (ammonium salts), and dough conditioners (oxidizing agents, e.g., ascorbic acid (E300)) <sup>(1,3,5)</sup>.</li> <li>- Can enhance yeast activity through addition of malt flour/malt extract, or other enzyme active preparations that produce fermentable sugars <sup>(1,5)</sup>.</li> </ul>
<b>Emulsifying agents</b> (monoglycerides (E471), esters from monoglycerides and diacetyltartaric acid (DATA esters; E472e), sodium or calcium stearyl-2-lactylate (SSL, E481 or E482), lecithin (E322)) <sup>(1)</sup> .	Lubricant/softener <ul style="list-style-type: none"> <li>- Influence is based on interactions with the starch-protein-fat-water components <sup>(1)</sup>.</li> <li>- It can improve the strength of the gluten network, rate of hydration, crumb structure, dough handling, slicing characteristics and gas retention and delay staling <sup>(1,4)</sup>.</li> </ul>
<b>Preservatives</b> (calcium propionate (E282), sorbic acid (E200), and vinegar)) <sup>(1)</sup> .	Shelf life <ul style="list-style-type: none"> <li>- Helps delay bread staling, control water activity and retard mold growth <sup>(1,3,4)</sup>.</li> <li>- Can affect yeast fermentation <sup>(1)</sup>.</li> </ul>

\*Adapted from: Collando-Fernandez , 2003 <sup>(1)</sup>; Goesaert et al, 2005 <sup>(2)</sup>; Moore, 2004 <sup>(3)</sup>; Mondal & Datta, 2008 <sup>(4)</sup>; Lai & Lin, 2006 <sup>(5)</sup>.

hydrolysis (Hoseney, 1998a). Starch, through its degradation products/sugars, also plays a role in the final crust colour of the bread through the Maillard reaction and its products. When alpha-amylase (i.e., endo-amylase) is added to the flour this causes the breakdown of damaged starch which generates low molecular weight dextrins and some maltose, which is a reducing sugar. In addition to the endo-amylase, endogenous beta-amylase produces maltose from low molecular weight dextrins, which then reacts with amino acids and results in a browning of the crust (Goesaert et al., 2005).

### *Proteins*

Proteins contained within the wheat flour include both non-gluten (~15-20 %) and gluten proteins (~80-85 %) (Hoseney, 1998d; Goesaert et al., 2005). The non-gluten proteins fall under the albumin and globulin classification as these are soluble in water and dilute salt solutions, respectively (Hoseney, 1998d; Goesaert et al., 2005). These proteins, which include enzymes and structural proteins, are located in the outer layer of the wheat kernel, and are lower in glutamic acid/glutamine and proline and are much higher in aspartic acid, arginine and lysine than the gluten proteins (Hoseney, 1998d; Goesaert et al., 2005). The gluten proteins (alcohol soluble prolamins) are the major storage proteins in wheat (Hoseney, 1998d; Goesaert et al., 2005; Wieser, 2007). Gluten proteins are high in glutamine (~35%), proline (14%) and hydrophobic amino acids (35%) (Hoseney, 1998b; Wieser, 2007). However, gluten has low levels of acidic and basic amino acids (Hoseney, 1998b; Wieser, 2007). Gluten is also low in cysteine (~2%) which is important for intra and inter-molecular disulphide bond formation (Wieser, 2007). Gluten is a complex quaternary structure comprised of two protein classes: gliadins and glutenins. The latter are also delineated as high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Hoseney, 1998b; Goesaert et al., 2005; Wieser, 2007). Gliadins are thought to contribute to dough viscosity/plasticity, whereas glutenins are cross-linked by disulphide bonds and contribute to dough elasticity (Butow et al., 2002; Goesaert et al., 2005; Wieser, 2007).

### *Non-starch polysaccharides (NSP)*

Non-starch polysaccharides (NSP) include cellulose,  $\beta$ -glucans and pentosans, which are found within the cell wall matrix (Eliasson & Larsson, 1993b). The dry matter weight of the

wheat endosperm cell wall is comprised of 75% NSP, with pentosans contributing ~85% (Goesaert et al., 2005). Pentosans are comprised of polymers of pentoses (mainly arabinose and xylose from arabinoxylans), which are five carbon monosaccharides (Eliasson & Larsson, 1993b). There are water soluble and water insoluble pentosans; water insoluble account for more than 60% of the total pentosans (Eliasson & Larsson, 1993b). Pentosans influence the water distribution in dough because of their strong water-holding capacity and are known to increase dough viscosity (Eliasson & Larsson, 1993b).

### *Lipids*

The lipids within wheat flour are classified by their association with the starch granule or not: starch lipids and non-starch lipids (Hoseney, 1998c). Non-starch lipids are further classified as nonpolar lipids (~60%), glycolipids (25%), or phospholipids (15%) (Hoseney, 1998c). The starch lipids are grouped into nonpolar (9%), glycolipid (5%), or phospholipid (86%) categories (Hoseney, 1998c). Nonpolar non-starch lipids have a detrimental effect on bread loaf volume; however, the polar lipids and glycolipids have been found to increase the loaf volume capacity at high concentrations, but decrease loaf volume at lower concentrations (Goesaert et al., 2005; Lasztity & Abonyi, 2009). It is postulated that polar lipids, mainly galactose-containing glycolipids, reinforce the gluten network through lipid-protein interactions (Lasztity & Abonyi, 2009).

### *Role of flour components in processing*

During dough development, gluten proteins are responsible for forming a continuous multiphasic viscoelastic network. Gluten makes up the network through disulphide bonding, and non-covalent interactions (i.e., hydrogen and ionic bonding, van der Waals, and hydrophobic interactions) (Salvador et al., 2006). During mixing, protein polymers become hydrated and randomly oriented. Continued mixing causes the polymers to re-orient within the directional shear, fostering increased protein-protein interactions to form a continuous gluten network leading to the dough's desired elasticity and extensibility (Lai & Lin, 2006; Salvador et al., 2006). This gluten network represents an insoluble protein phase within the dough.

Embedded within the gluten matrix is another phase comprised of intact and damaged starch granules, water, and water-soluble components such as pentosans (Jekle & Becker, 2011).



Lipid-gluten interactions form upon kneading to help stabilize the gas bubbles for greater gas retention within the gluten matrix (Jekle & Becker, 2011). Starch's role in dough formation is not entirely clear, but it is capable of absorbing large amounts of water (~46%), and upon heating the starch granules swell and gelatinize, reinforcing the gluten network (Petrofsky & Hosney, 1995; Goesaert et al., 2005). During gelatinization, heat causes the starch granules to experience irreversible changes to their ordered molecular structure, resulting in loss of birefringence, loss of X-ray diffraction pattern, absorption of water and swelling, and change in both shape and size (Eliasson & Larsson, 1993b; Goesaert et al., 2005). Then following gelatinization, leaching of amylose molecules from the starch granules occurs. Although the starch-protein interactions are still not fully understood, it is widely believed that they influence the rheological behaviour of the dough (Petrofsky & Hosney, 1995). Starches also contribute to crumb formation, colour, flavour and texture upon baking the dough into bread.

There is also a third phase within the dough, entrapped air and CO<sub>2</sub>. The CO<sub>2</sub> generated by yeast provides the leavening capacity that gives bread products their characteristic crumb structure (Campbell et al., 1998; Scanlon & Zghal, 2001). Doughs that have excellent gas retention properties result in breads with acceptable heights and textures and appealing structure (Stauffer, 2007).

### ***2.3.2 Role of water***

Hydration of the wheat flour is a prerequisite to dough formation. Water plays an important role within the complex dough system and in its conversion into bread. Water hydrates gluten proteins, starch and NSP within the flour and dissolves and disperses sugar and salt (Collando-Fernandez, 2003; Lai & Lin, 2006). Water also serves as a medium for both biochemical and chemical reactions, affecting the shelf life of the final baked product (Moore, 2004; Lai & Lin, 2006). The amount of water necessary for optimum dough formation is referred to as the flour's water absorption level, and depends on the quality and amount of protein, amount of starch and damaged starch, and NSP levels (Eliasson & Larsson, 1993a; Collando-Fernandez, 2003). Water absorption of flour can be measured using a farinograph. Water levels in the dough formulations can be adjusted depending on the flour to give consistent dough properties, hydration time and energy input needed for mixing (Farahnaky & Hill, 2007).

### **2.3.3 Role of salt**

Salt (e.g., NaCl) plays a critical role during dough and bread formation (Farahnaky & Hill, 2007; Miller & Hoseney, 2008). Salt modulates yeast fermentation, enhances product flavour, strengthens the gluten network (as measured by an increase in the storage modulus), increases the dough mixing time for increased protein-protein interactions, and acts as a preservative by decreasing water activity and prolonging shelf life (Moore, 2004; Miller & Hoseney, 2008; Belz et al., 2012). Altering the salt content in dough can impact the level of protein-protein interactions and the strength of the gluten network by changing the level of gluten hydration. Salt shields charged amino acids on the protein's surface reducing the thickness of the electric double layer, strengthening gluten interactions, and yielding a stronger network capable of retaining gas bubbles (Collando-Fernandez, 2003). Decreased levels of salt in the dough result in the protein becoming less shielded to enable a greater amount of protein-water interactions and, thus a weaker gluten network. Without adequate levels of salt, there will be insufficient formation of the gluten network (dough structure), and increased activity of the yeast, which leads to poor bread quality in terms of texture, volume, flavour and colour (Farahnaky & Hill, 2007). The range of salt used within the bread formulation is ~1-2% on a flour weight basis (Collando-Fernandez, 2003).

### **2.3.4 Role of yeast**

Yeast (*Saccharomyces cerevisiae*) is a leavening agent that converts simple fermentable carbohydrates to CO<sub>2</sub> and alcohol; it is the release of CO<sub>2</sub> that produces the leavening action in bread (Lai & Lin, 2006). These simple carbohydrates can either be added to the formulation, or formed through the hydrolysis of starch using enzymes (Lai & Lin, 2006). Yeast is only active in the temperature range of 0-55°C; however, the most favourable temperature for fermentation is between 27-38°C, with the greatest activity occurring at 35°C (Lai & Lin, 2006). During the fermentation process, the activity of the yeast expands the bubbles in the dough and also creates fermented yeast flavours (Lai & Lin, 2006). Specific compounds formed during yeast fermentation are organic acids, alcohols, aldehydes, esters, and ketones; some of these compounds are volatilized when the bread is baking, some lead to further reactions, but most add to the flavour and odour of the product (Lai & Lin, 2006). Some of the compounds created can

act as dough conditioners and serve to increase the dough's extensibility by relaxing the gluten (Lai & Lin, 2006).

## **2.4 BREADMAKING PROCESS**

The breadmaking process typically follows three major operations: dough formation, fermentation and baking (Hoseney, 1998e).

### ***2.4.1 Dough formation***

The formation of dough begins with the mixing process, which combines all the ingredients into a more uniform dough, encourages dissolution and hydration of ingredients, distributes the yeast evenly throughout the dough, incorporates air bubbles within the dough to provide gas nuclei for CO<sub>2</sub>, develops the gluten network, and ultimately forms a viscoelastic dough suitable for further production (Autio & Laurikainen, 1997; Hoseney, 1998e, Lai & Lin, 2006; Marsh & Cauvain, 2007). During mixing, proteins become hydrated, partially unravel and re-orient within the direction of shear to develop strong protein-protein interactions stabilized by disulphide bonds, hydrogen bonding, and hydrophobic interactions to form a viscoelastic network (Hoseney, 1998e; Letang et al., 1999; Lai & Lin, 2006; Belz et al., 2012). Parameters such as the mechanical energy applied during the stretching and shearing process, mixing time, shear rate, temperature and flour:water ratio are all essential to develop the optimum gluten network while avoiding the occurrence of sticky dough (Letang et al., 1999). When over-mixing occurs, the gluten polymers over extend causing loss in elasticity and depolymerize by breaking disulphide bonds (Autio & Laurikainen, 1997; Letang et al., 1999; Lai & Lin, 2006). Under-mixing of the dough results in an uneven distribution of the proteins and starches to give a poorly developed gluten network.

### ***2.4.2 Fermentation***

Fermentation is the next stage in the breadmaking process, and can be divided into additional sub-steps: fermentation, punching, dividing, molding, panning and proofing (Collando-Fernandez, 2003). Initially yeast cells (*Saccharomyces cerevisiae*) adapt to the dough, and switch from aerobic fermentation to anaerobic when O<sub>2</sub> becomes depleted (Elmehdi et al., 2003). The change in fermentation leads to the production of CO<sub>2</sub> and ethanol, which causes

changes to the physical properties of the dough (Hoseney, 1998e; Collando-Fernandez, 2003). The latter involves the gluten matrix becoming more elastic, allowing it to withstand the expansion of gas cells (Collando-Fernandez, 2003; Lai & Lin, 2006; Belz et al., 2012). If under-fermentation of the dough occurs, then the resulting texture of the loaf will be coarse and if over-fermented, the texture will become sticky (Lai & Lin, 2006). Next the dough undergoes punching, which is a process of deflating the dough to allow for the expulsion of the CO<sub>2</sub>, redistribution of the yeast and relaxation of the gluten (Hoseney, 1998e; Lai & Lin, 2006). During punching, the dough is pulled up on all sides, then folded over the center, and then finally pressed down (Lai & Lin, 2006). The dough is then divided, rounded, molded/sheeted and panned before entering the proofer. Dividing involves the creation of dough pieces of similar weight, whereas rounding involves shaping these pieces into smooth balls (Collando-Fernandez, 2003; Lai & Lin, 2006). During the rounding stage, an intermediate proofing step may be used (10-20 min) to allow for relaxation of the gluten polymers to make shaping of the dough easier (Hoseney, 1998e; Collando-Fernandez, 2003; Moore, 2004; Lai & Lin, 2006). Sheeting and molding involves the expulsion of gas and flattening of the dough balls, then curling the dough into cylinders, and seam sealing the dough with sheeting rollers (Hoseney, 1998e; Moore, 2004). The process of sheeting has an effect on reducing the amount of gas and reorganizing the protein network within the dough (Autio & Laurikainen, 1997). Panning involves the dropping of the dough into the pans on a conveyor (Moore, 2004). Proofing is considered the final fermentation step, which involves a resting period to allow the yeast to generate more CO<sub>2</sub> and ethanol to cause the dough to rise (Moore, 2004; Lai & Lin, 2006). Proofing is usually carried out at a temperature of 30-35°C at a relative humidity of 85% for 55-65 min (Hoseney, 1998e). The amount of CO<sub>2</sub> retained in the proofed loaf is highly dependent on the quality of the gluten network that has been formed during dough formation and fermentation.

### ***2.4.3 Baking***

The final stage in the breadmaking process is baking, which transforms the viscoelastic dough into a solid springy loaf with an outer crust and internal porous crumb structure. During baking, a) dough volume rapidly rises as gas bubbles expand, b) starch partially gelatinizes, a process whereby starch granules swell and lose birefringence; c) proteins denature, cross-link and aggregate together to form a solid structure; d) fat crystals (if added to the dough) melt and

become incorporated into the bubble interface to prevent rupturing; and e) crust colour and flavours develop due to Maillard browning (i.e., non-enzymatic browning involving a chemical reaction between an amino acid and a reducing sugar when exposed to a sufficiently high temperature) (Autio & Laurikainen, 1997; Hoseney, 1998e; Lai & Lin, 2006; Belz et al., 2012). Once the dough has been transformed into a springy loaf the bread is then cooled, sliced and packaged (Collando-Fernandez, 2003; Moore, 2004).

## 2.5 WHEAT QUALITY

The characteristics that determine whether a wheat flour is suitable for breadmaking depend on the cultivar of the wheat, the location where the wheat is grown, the growing conditions, and the type and extent of milling of the wheat; all these factors have an impact on the composition of the flour (Lai & Lin, 2006; Lasztity & Abonyi, 2009). The cultivars of *Triticum aestivum* L. are divided into either soft or hard wheat cultivars (Veraverbeke & Delcour, 2002; Lai & Lin, 2006; Wheat Marketing Center, 2008; Delcour et al., 2012). Hard and soft wheat cultivars are classified based on kernel strength and the amount of force necessary to crush the kernels (Delcour et al., 2012). The hard wheat cultivars, having a higher protein content (~10-14%), are capable of producing highly elastic dough with extensibility and are typically utilized for yeast leavened products such as breads (Payne, 1987; Lai & Lin, 2006; Wheat Marketing Center, 2008; Delcour et al., 2012). In contrast, the soft wheat cultivars, having a lower protein content (~8-10%), create more extensible doughs and are typically utilized for producing cookies and pastries (Payne, 1987; Lai & Lin, 2006; Wheat Marketing Center, 2008; Delcour et al., 2012). The balance between elasticity and extensibility is controlled by genetics and can differ greatly between wheat cultivars, influencing which wheat cultivars can be used for certain food products (Payne, 1987).

Breadmaking performance and dough rheology is highly dependent on the differences in the protein quantity and composition/quality. The quantity and composition/quality of flour protein gives an indication of whether the flour will be strong enough to create an elastic dough translating into a low density loaf with a fine and uniform crumb structure (Sliwinski et al., 2004; Goesaert et al., 2005; Lai & Lin, 2006). To determine wheat quality, a number of protein characteristics are investigated: total protein, gliadin-to-glutenin ratio, and glutenin content (especially with respect to the amount of low molecular weight and high molecular weight

glutenins and their molecular size distributions) (Sliwinski et al., 2004). A breakdown of some of the factors that affect dough rheological properties is represented in Table 2.2.

### **2.5.1 Gluten proteins: *glutenin & gliadin***

Making up ~80-85% of the total wheat proteins, the gluten proteins are categorized under the prolamin class in the Osborne classification scheme (Veraverbeke & Delcour, 2002; Goesaert et al., 2005). The gluten proteins are insoluble in both water and dilute salt solutions; however, they are partially soluble in alcohol or dilute acidic or alkaline solutions (Goesaert et al., 2005). Gliadins are a heterogeneous mixture of non-cross-linked proteins soluble in aqueous alcohols (70% ethanol) with molecular weights varying between 30,000 - 80,000 Da and can occur biochemically in three types ( $\alpha$ ,  $\gamma$ , and  $\omega$ ) (Veraverbeke & Delcour, 2002; Sliwinski et al., 2004; Goesaert et al., 2005). A comparison of the  $\alpha$ - and  $\gamma$ -type gliadins to the low molecular weight glutenin subunits show that they are related through the amino acid sequences classification of being 'sulphur-rich prolamins' (Veraverbeke & Delcour, 2002). There are six cysteine residues in the  $\alpha$ -type gliadins and eight cysteine residues in the  $\gamma$ -type gliadins; these cysteine residues result in the intra-chain disulphide bonds within these types of gliadins (Veraverbeke & Delcour, 2002). On the other hand, the  $\omega$ -type lack cysteine residues and are low in methionine, giving them the classification of 'sulphur-poor prolamins' (Veraverbeke & Delcour, 2002).

The glutenins are made up of a heterogeneous mixture of polymers with molecular weights ranging from 80,000 Da to several millions. These large sizes are the reason that glutenins are deemed unextractable; however, the glutenin subunits can be extracted upon the breaking of inter-chain disulphide bonds (Veraverbeke & Delcour, 2002; Sliwinski et al., 2004; Goesaert et al., 2005). Once the glutenin polymers have been treated with a reducing agent such as  $\beta$ -mercaptoethanol or dithiothreitol, the smaller polymers are extractable in aqueous alcohol and the larger polymers are extractable in dilute acid or alkali solutions (Veraverbeke & Delcour, 2002; Sliwinski et al., 2004; Goesaert et al., 2005). It is difficult to determine the structure/function relationships in gluten because extraction of glutenin polymers alters structure (Goesaert et al., 2005). The glutenins can be sub-divided into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). The HMW-GS have molecular weights ranging from 65,000 - 90,000 Da. The LMW-GS can further be broken down into three types (B-, C-, and D-type) and have molecular weights ranging from 30, 000 -

Table 2.2 Factors affecting dough rheology.

Dough rheological properties		
Composition of dough	Gluten quantity and quality	Processing parameters
<ul style="list-style-type: none"> <li>• Flour</li> <li>• Damaged starch</li> <li>• Pentosans</li> <li>• Yeast</li> <li>• Water</li> <li>• Salt (NaCl)</li> <li>• Shortening/fat</li> <li>• Sugars</li> <li>• Emulsifiers</li> <li>• Preservatives</li> <li>• Yeast food</li> </ul>	<ul style="list-style-type: none"> <li>• Glutenin               <ul style="list-style-type: none"> <li>• HMW-GS</li> <li>• LMW-GS</li> <li>• HMW-GS/LMW-GS ratio</li> </ul> </li> <li>• Gliadin</li> <li>• Gliadin/glutenin ratio</li> <li>• Size distribution</li> <li>• Structure</li> </ul>	<ul style="list-style-type: none"> <li>• Type of mixer</li> <li>• Mixing speed</li> <li>• Mixing time</li> </ul>

\*Abbreviations: HMW-GS (High molecular weight glutenin subunits), LMW-GS (Low molecular weight glutenin subunits).

60,000 Da (Goesaert et al., 2005). The LMW-GS are similar to the gliadins in that they are ‘sulphur rich’, and capable of forming intra-chain disulphide bonds (Veraverbeke & Delcour, 2002). However, the LMW-GS differ from the gliadins in their capability to also form inter-chain disulphide bonds with the HMW-GS (Veraverbeke & Delcour, 2002). Both the gliadins and glutenin subunits are high in glutamine and proline (Veraverbeke & Delcour, 2002). The HMW-GS tend to have higher amounts of glycine than the gliadins and the LMW-GS (Veraverbeke & Delcour, 2002).

A high quantity and good quality of gluten proteins making up the total protein content of the wheat flour is important for the manufacture of high quality bread. Numerous researchers have found that wheat flour performance for breadmaking is linearly related to flour protein content, and ultimately the gluten fraction, because gluten increases more than the non-gluten protein fraction with increasing total protein (Finney & Barmore, 1948; Veraverbeke & Delcour, 2002). This linear relationship, however, was found to be dependent on wheat cultivar, which reveals that the quality of the gluten protein is also a factor (Finney & Barmore, 1948; Veraverbeke & Delcour, 2002). Gluten imparts dough properties of extensibility, viscosity,

elasticity and cohesiveness, as well as contributing to water absorption; the extent of these characteristics highly depends upon the quality and ratio of the gliadins and glutenins that make up gluten as each serve a different purpose (Goesaert et al., 2005; Joye et al., 2009).

Gliadins play a role in viscosity and extensibility, and as such act as a plasticizer within the dough system (Oates, 2001; Goesaert et al., 2005; Joye et al., 2009; Delcour et al., 2012). Glutenin proteins form a continuous network through inter-molecular disulphide bonding, giving the gluten network elasticity and cohesiveness (He et al., 1992; Joye et al., 2009). The ratio of gliadins to glutenins (Gli/Glu ratio) has a significant effect on dough formation properties (dough stability, development time, viscosity) as well as the final bread product (bread volume and crumb firmness) (Barak et al., 2013). When there is a high ratio of Gli/Glu, then the dough's resistance to extension decreases and extensibility increases to create a weak dough because of the larger amount of gliadins weakening the interactions between the glutenin chains; when the ratio is low, increased elasticity is imparted to the dough from the glutenin polymers, allowing for greater loaf volume upon expansion of the gas (Khatkar et al., 1995; Sliwinski et al., 2004). Therefore, a necessity for the production of quality bread requires a balance between dough extensibility from the gliadins and elasticity/strength from the glutenins (Veraverbeke & Delcour, 2002; Goesaert et al., 2005).

The glutenin fraction plays a larger role, when compared to the gliadin fraction, in determining the quality differences in bread (Khatkar et al., 1995; Janssen et al., 1996; Veraverbeke & Delcour, 2002; Sliwinski et al., 2004; Goesaert et al., 2005; Lasztity & Abonyi, 2009). In addition to a higher quantity of glutenins than gliadins being important for the proper dough formation, composition of the glutenin subunits is also highly important. The differences in functionality arising from the glutenin fractions comes from differences in the composition (i.e., amino acids), structure, and/or size distribution of the polymers (Goesaert et al., 2005). Any differences in the amino acid composition of the glutenin subunits cause changes in the potential for non-covalent interactions, both between and within the glutenin subunits, and this influences the elasticity of the glutenin polymer network (Goesaert et al., 2005). All wheat cultivars contain from 3 to 5 different HMW-GS and between 7 and 16 LMW-GS (Payne, 1987; Oates, 2001; Goesaert et al., 2005). In general, wheat flours that form strong doughs with long mixing times contain relatively large amounts of glutenins of large size (Sliwinski et al., 2004).



## 2.6 SODIUM REDUCTION IN DOUGH

Beyond NaCl's many roles during the baking process and its relationship with wheat flour quality, salt reduction can have a major impact on the handling of dough (stickiness) and the final loaf attributes.

### 2.6.1 Dough stickiness

Dough stickiness is an extremely complex issue and the lack of objective methods to measure this phenomenon has hindered advances in this research area (Chen & Hoseney, 1995a; Hoseney & Smewing, 1999; Adhikari et al., 2001). Dough stickiness results when the adhesive forces (interactions between the dough and mixing surfaces) are high and cohesive forces (interactions within the dough) are low (Adhikari et al., 2001; van Velzen et al., 2003). Occurrence of sticky dough is affected by processing parameters such as level of hydration, mixing conditions (temperature, time, procedure) and formulation (e.g., flour, salt, etc.), which all affect gluten network formation (Miller & Hoseney, 2008; Beck et al., 2012a). The use of the 1B/1R chromosome translocation to confer disease resistance in wheat breeding programs resulted in sticky dough problems in industrial bakeries. Reduced dough strength, increased water absorption and intolerance to over mixing were also seen in these cultivars, pointing to issues in dough handling performance (Dhaliwal et al., 1990; Hoseney & Smewing, 1999; Adhikari et al., 2001). Other factors such as wheat flour extraction process (e.g., milling), amount of water-soluble pentosans, differences in protein composition and quality,  $\alpha$ -amylase and proteolytic enzyme activities, also have an effect on dough stickiness making the solution to the problem highly complex (Chen & Hoseney, 1995a; Hoseney & Smewing, 1999). A means to overcome sticky dough is through the use of NaCl at ~1.8-2.1% on a flour weight basis (Farahnaky & Hill, 2007). It is still not fully understood whether the root cause of sticky dough is a single factor or multiple factors acting in combination. When reducing NaCl levels in bread dough formulations, the factors described above can play an increasing role in causing dough stickiness since the presence of a weak gluten network can no longer compensate for the increased water mobility within the dough.

### ***2.6.2 Stickiness component within the 1B/1R wheat cultivars***

Gore (1991) found flour extraction affected dough stickiness through its effect on flour quality. Flour extraction would affect the quality of the wheat flour through the quantity/quality of the protein as well as the amount of damaged starch present. Much of the research done on sticky dough has been carried out as the result of the use of the 1B/1R translocation wheat cultivars. These cultivars have the short arm of the 1B chromosome for wheat replaced by the short arm of the 1R chromosome from rye, this results in higher yields and good resistance to stem, leaf, and stripe rusts (Dhaliwal et al., 1988). Although dough stickiness has been found in wheat cultivars containing the 1B/1R translocation, a strong cause-and-effect relationship is complicated by numerous other factors that can influence dough handling/stickiness (Dhaliwal et al., 1988; Barbeau et al., 2003). Different hypotheses have been proposed as to the cause of the sticky dough in some of the wheat cultivars containing the 1B/1R translocation. For instance, Zeller et al. (1982) implicated water soluble pentosans as they affect the amount of water that is absorbed by the dough because of their ability to bind water through hydrogen bonding. However, Dhaliwal et al. (1988) found that there was a high pentosan content in certain wheat flours exhibiting no stickiness and flour with a low content of pentosans exhibiting high stickiness, suggesting that pentosans are not a primary cause of stickiness. Henry et al. (1989) stated that in translocated wheat cultivars there were higher amounts of  $\beta$ -glucan in the endosperm of those cultivars producing sticky dough, and therefore  $\beta$ -glucan may be a contributing factor in dough stickiness.

In a review on dough stickiness in the 1B/1R wheat cultivars, Barbeau et al. (2003) provided a summary of three potential hypotheses that have the greatest support based on prior research for the cause of dough stickiness. First, rye secalin proteins coded on the short arm of the 1R chromosome replace the LMW-GS 1B chromosome, resulting in the loss of the LMW-GS and the formation of a weaker gluten network (Graybosch et al., 1993; Barbeau et al., 2003). The second hypothesis attributes the stickiness to the higher content of the cell wall polysaccharides (pentosans and  $\beta$ -glucans) because of their effect on water absorption (Henry et al., 1989; Eliasson & Larsson, 1993a; Barbeau et al., 2003). The third hypothesis, presented by Chen and Hoseney, (1995b) was based on the finding that in some of the 1B/1R translocation cultivars with optimal mixing and appropriate water absorption dough stickiness was still evident. Therefore, they stated there may be a water-soluble compound causing sticky dough, which they

identified as a ferulic acid ester of hexose-containing polysaccharide (Chen & Hoskeney, 1995b; Barbeau et al., 2003).

The proper formation of an evenly dispersed gluten network is a crucial element in forming the structure of bread and the rheological properties of the dough. In the literature, it is agreed that anything that affects the rheological properties of the dough can have an effect on dough stickiness, therefore making the gluten network a primary factor to examine when studying dough stickiness. Factors (i.e., mixing conditions, water mobility, and temperature) that affect the bonding between the gluten proteins through non-covalent bonds (hydrogen bonds, ionic bonds and hydrophobic interactions) affect the formation of the gluten network because those bonds are necessary for aggregation of the gliadins and glutenins, which determine the structure and physical properties of dough (Wieser, 2007).

### ***2.6.3 Stickiness as a result of processing parameters***

Mixing conditions and water content play a major role in determining the quality of the protein-protein interactions which occur. Mixing conditions (i.e., mixer type, rotation speed, mixing time and water content) affect the gluten network by either: a) increasing the level of protein alignment which leads to a greater amount of protein-protein interactions, bonding and structure development within the dough; or b) disrupting the bonds between the proteins (over-mixing), which results in structure breakdown and a sticky dough (Singh et al., 2002). An optimal mixing time is related to optimum baking performance and good crumb structure (Zghal et al., 1999). Mixing time is also considered to be positively correlated with the polymeric protein composition, the ratio between glutenins and gliadins, flour quality and amount of other ingredients (e.g., salt) (Letang et al., 1999; Dobraszczyk & Morgenstern, 2003; Angioloni & Rosa, 2005). As previously discussed, initial mixing serves to hydrate the flour components and at this point the glutenin proteins are folded with random orientation of the protein chains. As mixing time is increased, the glutenin polymers align with the shear and stretching forces of the mixer, followed by the formation of cross-links between the proteins (Letang et al., 1999). A large number of protein-protein interactions ensues to create a gluten network that increases dough strength. A strong dough is a non-sticky dough (Letang et al., 1999). Once the dough has been mixed beyond its optimum mixing time (determined by the maximum Barbender consistency of the dough measured by either a mixograph or farinograph), the disulphide bonds

that hold the glutenin polymers together can be broken leading to depolymerization and the creation of lower molecular weight glutenin subunits. The latter leads to a decrease in Barbender consistency and thus a sticky dough because of the presence of smaller protein chains (Letang et al., 1999).

#### ***2.6.4 Stickiness due to level of damaged starch***

Another factor that can influence the hydration of the gluten network is the level of damaged starch within the flour. Native starch granules are thought to not contribute to dough stickiness during the dough formation stage; however, damaged starch does contribute to dough stickiness. Starch granules become damaged during milling of wheat into flour. Typically, ~5-8% of starch is physically damaged during the milling process; although minor in quantity, this level of damaged starch can play a significant role in water mobility (Van Der Borgh et al., 2005). The amount of damaged starch is dependent upon the degree of grinding and the hardness of the wheat (Van Der Borgh et al., 2005). Damaged starch's contribution to dough stickiness is not fully understood. Within the literature, it is hypothesized that during the mixing stage the protein and the starch compete for water, and this competition is dependent upon the level of damaged starch present (Eliasson & Larsson, 1993a; Butow et al., 2002). Damaged starch has lost its birefringence because of the physical changes to the crystalline structured region; because of the latter, damaged starch absorbs more water than native starch (Goesaert et al., 2005; Van Der Borgh et al., 2005). Starch provides support to the dough structure, whereas damaged starch is more susceptible to enzymatic hydrolysis; which can lead to some loss of structure as the starch is converted to sugars resulting in the dough becoming sticky (Goesaert et al., 2005; Van Der Borgh et al., 2005; Wheat Marketing Center, 2008). Jekle and Becker (2011) proposed that when there is excess water present within the dough, the hydration of starch granules may increase causing the granules to expand or congregate. This increase in starch granule size and aggregation would impede the gluten network development due to the barriers created by starch granules. The increase in water uptake by the starch granules will also create a protein dilution effect according to Jekle and Becker (2011).

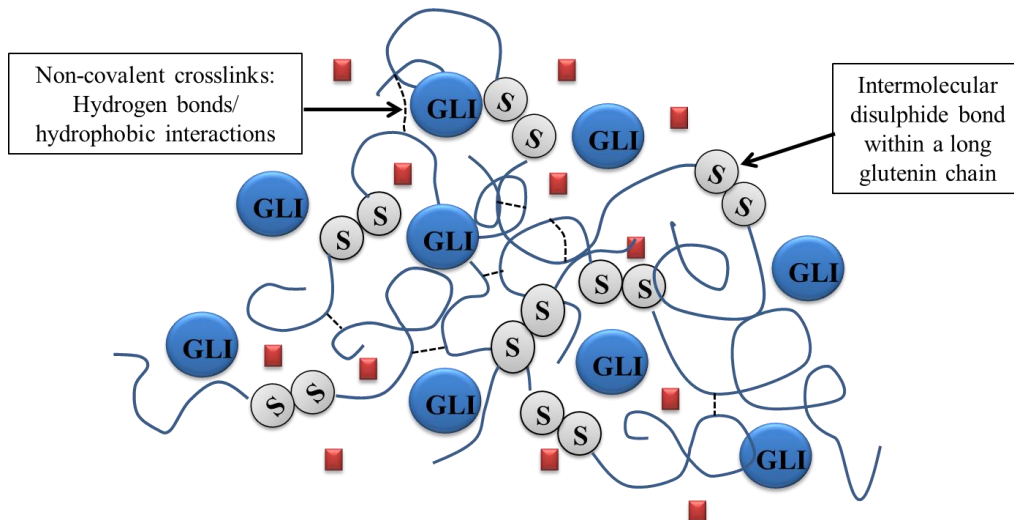
### ***2.6.5 Stickiness due to water mobility/protein hydration***

When Chen and Hosney (1995a) used flour that had a propensity to form sticky dough and decreased the amount of water added, a less sticky dough was created. Jekle and Becker (2011) found that with increased water content, dough stickiness significantly increased and the storage modulus decreased indicating that there was a loss in structure. They concluded that the excess water created an increased contact area between the dough and the stickiness fixture and thus increased the adhesion force between surfaces. van Velzen and others (2003) explained increased adhesion as the increased stickiness resulting from the degree of hydration of the gluten protein. As the hydration increases the mobility of the proteins increases and thus gluten proteins migrate to the upper dough layers from the bulk. This then decreases the starch content at the upper layers of the dough and the starch may be pushed to deeper layers where the starch absorbs the excess water and swells. Movement of the gluten proteins to the surface and the decrease in the starch content at the surface of the dough led van Velzen and others (2003) to conclude that it is the gluten protein hydration that plays an important role in dough stickiness. This explanation was reached through the use of attenuated total reflectance and Fourier transform infrared spectroscopy of the dough's surface. They found that at higher hydration states of the gluten protein there were higher amide intensities at the surface as opposed to the starch and water. Jekle and Becker (2011) noted that when there is a greater amount of water in the dough, the excess hydration will increase the inter-molecular space between the protein phase and the starch phase. Jekle and Becker (2011) examined the effect of water addition on the dough structure through the use of confocal laser scanning microscopy and showed a transition from a gluten network that is not fully formed/ uniformly distributed with the lowest water addition to a gluten network that becomes uniformly distributed and properly formed with more of an optimal water addition (water level necessary for maximum dough consistency – near the 500 Barbender Unit line) to a gluten network that becomes disrupted and unevenly distributed (the proteins become more aggregated and space is taken up by the water, starch granules and air bubbles) with the addition of excess water. Despite this, it is not fully understood whether it is the effect of the water's interaction with the protein phase or the starch phase or both that results in dough stickiness, but it is generally agreed upon that water and the mobility of water affects dough stickiness. This effect of water on dough stickiness can be overcome, to an extent, by the addition of salt.

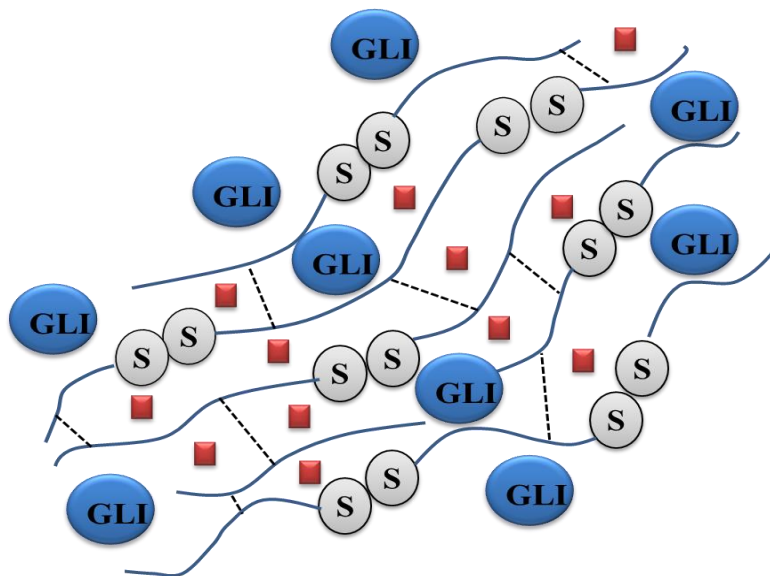
### ***2.6.6 Prevention of stickiness with sodium chloride (salt)***

Among its multiple roles in the breadmaking, salt is important for prevention of dough stickiness. Salt is used within the dough formulation to induce protein-protein interactions to aid in the creation of a strong gluten network. Salt increases the dough development time, dough stability, mixing tolerance, resistance to extension, extensibility, gelatinization temperature, and peak viscosity; on the other hand, it decreases water absorption as determined by farinograph measurements (Hlynka, 1962; Linko et al., 1984; Butow et al., 2002; Farahnaky & Hill, 2007). The increase in the aforementioned dough characteristics indicates that the salt creates stronger dough and therefore a well-structured gluten network, which will not be sticky. In the flour-water system (pH of ~6.0), the gluten proteins are below their isoelectric point, which is ~ pH 7.5, and hence they carry a net positive charge (Gennadios et al., 1993; Miller & Hoseney, 2008). This results in repulsion between the gluten proteins and faster protein hydration, resulting in shorter mixing time because proteins are kept from interacting, creating a weaker sticky dough (Miller & Hoseney, 2008). Because gluten proteins contain 35% hydrophobic amino acids, 35% hydrophilic, and 7% charged amino acids it is apparent that hydrophobic interactions between the proteins play a major role in the formation of the gluten network (Preston, 1989; He et al., 1992; Butow et al., 2002; Miller & Hoseney, 2008).

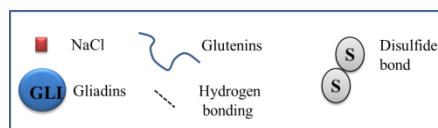
Once salt is added, it acts to shield the charges on the protein enabling the proteins to interact/aggregate through hydrophobic interactions leading to a decrease in the level of hydration of gluten proteins and a strengthening of the gluten network (Figure 2.1A, B) (Preston, 1989; Butow et al., 2002; Miller & Hoseney, 2008). This charge shielding increases the amount of protein-protein interactions through hydrophobic interactions, and decreases the amount of water-protein interactions. Salt occupies the sites originally taken up by the water hydrating the gluten proteins creating a more structured and less soluble protein network (Butow et al., 2002; Miller & Hoseney, 2008). Therefore, this results in an increase of unabsorbed water present as a result of increased protein-protein interactions within the gluten structure (Hlynka, 1962; Butow et al., 2002). Removing salt from the dough formulation results in weak and sticky dough as a result of poor gluten network formation caused by increased water-protein interactions and decreased protein-protein interactions (Figure 2.1C, D).



(A) Beginning of  
mixing with salt



(B) Protein alignment due to  
mixing



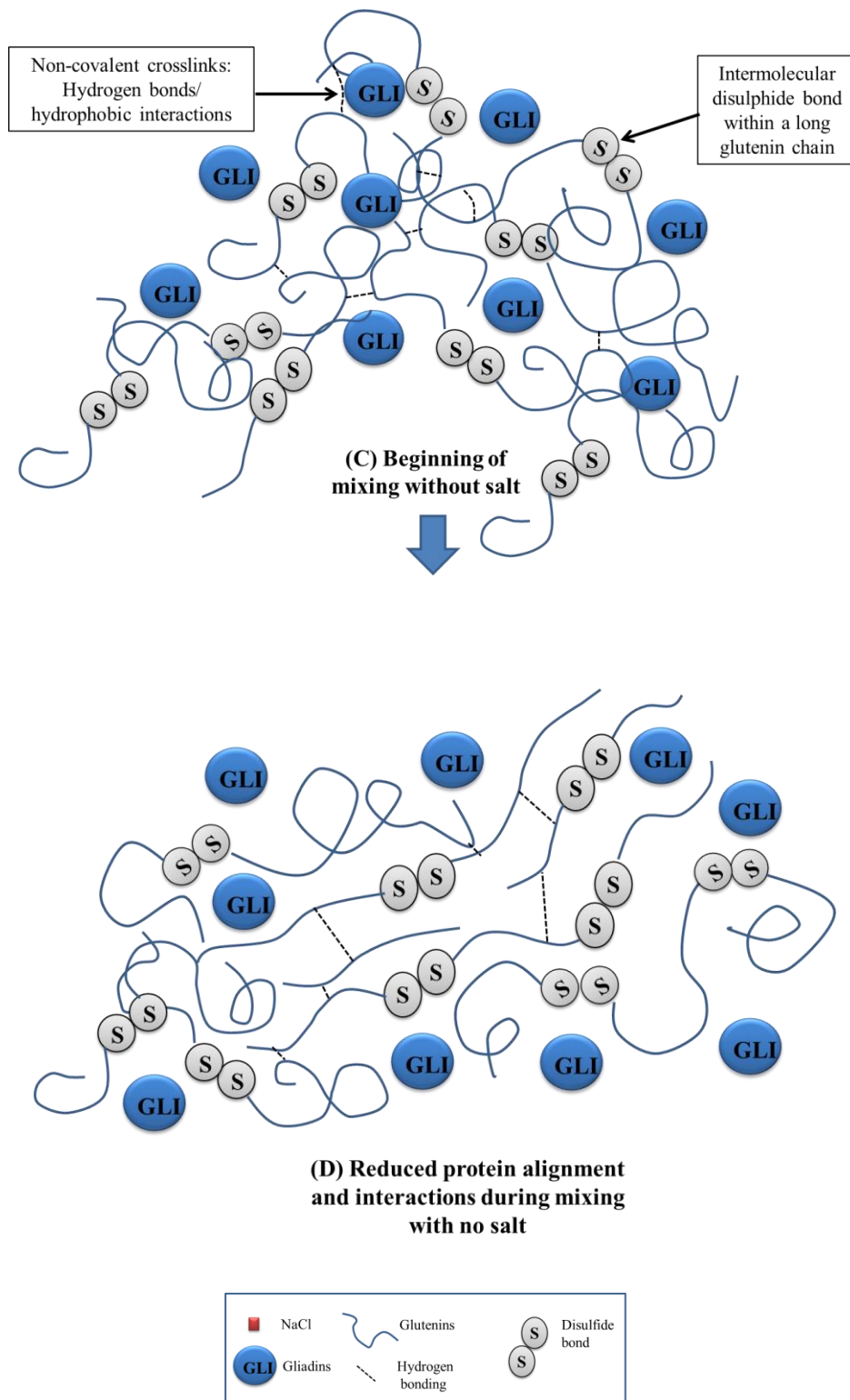


Figure 2.1 Depiction of the gluten network formation during mixing conditions with and without sodium chloride. Forming a strong gluten network and non-sticky dough in situation (A) & (B) and a weak gluten network and sticky dough in situation (C) & (D).



### ***2.6.7 Effect on gluten network formation***

Salt influences the physical requirements needed for proper dough development. When salt is removed from the formulation, the processing parameters must be altered because salt affects the water absorption of the flour, the mixing time, the mixing intensity, and the relaxation time needed (Belz et al., 2012). As discussed above, salt enhances the interaction between the gluten proteins creating a uniformly structured network as seen in Figure 2.1 (A & B). When the salt is removed, there is an increase in water-protein interactions and fewer protein-protein interactions resulting in the formation of a weak network as seen in Figure 2.1 (C & D). Through the use of confocal laser scanning microscopy, Beck et al. (2012a) were able to see that with a reduction in salt the gluten protein network changed from a structure with elongated protein strands to a network with less connected protein particles. Similar visual results of a decrease in the structured protein network with sodium reduction were also seen by Lynch et al. (2009) and McCann and Day (2013). The reduced protein-protein interactions are in part due to the loss of the electrostatic shielding effect of the salt inducing increased charge repulsion within the gluten network, and in part to increased protein hydration (McCann & Day, 2013).

For a stable dough with a strong gluten network, experimental results indicate a decreased water absorption (as measured with a farinograph), increased peak time/dough development time, decreased mixing tolerance index (MTI) value, increased dough stability time, increased resistance to extension, and increased extensibility; all are indicative of greater amounts of protein-protein interactions (Hlynka, 1962; Salovaara, 1982a; Linko et al., 1984; Letang et al., 1999; Kaur et al., 2011; Belz et al., 2012). In contrast, salt reduction within the dough formulations had been shown to lead to a weaker gluten network being formed. This weak network is evident by an increase in water absorption of the dough, decreased peak time/dough development time, increased mixing tolerance index or dough breakdown, decreased dough stability, decreased resistance to extension, and decreased extensibility, all of which translate into a sticky dough (Lynch et al., 2009; Belz et al., 2012; McCann & Day, 2013).

### ***2.6.8 Effect on final bread product***

The removal/reduction of salt from the dough formulation also has a negative impact on the bread's final quality from the visual appearance to the flavour and shelf life. Because salt impacts the formation of the gluten network and the activity of yeast, it also influences the crust

texture, colour, and flavour (Belz et al., 2012). With less salt there is more yeast activity, which results in decreased levels of free reducing sugars for the Maillard reaction to create the desired crust colour and flavour (Belz et al., 2012). Czuchajowska et al. (1989) reported that bread made without salt had a lighter crust colour due to the decreased Maillard reaction products resulting from the salt reduction. Lynch et al. (2009) found that bread without salt staled quicker than bread containing salt, and they concluded that because salt plays a role in controlling water mobility (through increased ordering of the gluten network) and reducing the rate of water migration from the crumb to the crust, there is greater water migration and thus staling. They also found that bread prepared without salt had an uneven crumb structure due to a weaker and less structured gluten network (Lynch et al., 2009). In regard to sensory attributes, Lynch et al. (2009) found that bread without salt was described as having a high “sour/acidic”, “sour dough”, and “yeasty” flavour. McCann and Day (2013), Czuchajowska et al. (1989), and He et al. (1992) found that loaf volume decreased with decreasing salt content. In contrast, Lynch et al. (2009) and Beck et al. (2012b) found an increase or no change in loaf volume with the reduction of salt. The differences between these studies could be due to differences in the processing parameters (i.e. mixing conditions: speed, time, energy input, temperature; mixer style; fermentation/proofing time; baking temperature and time) of the bread, the formulation (i.e., amount of yeast used, use of shortening or not) and protein quantity/quality of the flour.

## **2.7 STRATEGIES USED TO LOWER SODIUM**

To combat the effects of sodium reduction on dough handling and stickiness, various approaches have been investigated to improve gluten network formation.

### ***2.7.1 Effect of replacement of sodium with alternative salts***

Research has examined methods to overcome sticky dough with the removal/reduction of salt. The most researched strategy examines the replacement of the sodium ion ( $\text{Na}^+$ ) with potassium ( $\text{K}^+$ ), calcium ( $\text{Ca}^{2+}$ ), and magnesium ( $\text{Mg}^{2+}$ ) (to name a few) to remediate the problem of sticky dough and maintain ideal dough rheology, while at the same time reducing the levels of sodium within the bread. The effect of different cations is related to their position in the lyotropic series, also known as the Hofmeister series, which ranks ions based on ability to cause protein aggregation or dissociation (Salovaara, 1982a; Preston, 1989; Miller & Hoskeny, 2008).

Within the series, both anions and cations are ranked in order of most stabilizing to destabilizing (He et al., 1992; Miller & Hoseney, 2008). Stabilizing ions lead to less hydration, more structure and decreased protein solubility, whereas destabilizing ions lead to more hydration and increased protein solubility, thus affecting both hydrophobic interactions and hydrogen bonding (Preston, 1989; Butow et al., 2002; Miller & Hoseney, 2008). The ranking of most to least stabilizing cations are:  $\text{NH}_4^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ = \text{Na}^+ > \text{H}^+ > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{Al}^{3+}$  (He et al., 1992; Miller & Hoseney, 2008). Therefore, it would be expected that with the use of a stabilizing cation there would be increased protein-protein interactions to promote the formation of a stronger gluten network and thus a non-sticky dough. It appears from the literature that  $\text{K}^+$  is the best option for maintaining similar dough rheology to doughs containing sodium chloride since  $\text{K}^+$  is equivalent to  $\text{Na}^+$  in the lyotropic series. However, this replacement comes with the significant challenge of a metallic/bitter flavour (Salovaara, 1982a; Miller & Hoseney, 2008; Belz et al., 2012).

The farinograph is the most common analytical technique used to examine dough rheology as it provides information on dough water absorption, dough development time/peak time (amount of time required for dough to reach maximum consistency), mixing tolerance index (measure of gluten breakdown leading to dough softening) and dough stability (Salovaara, 1982a; Letang et al., 1999; Kaur et al., 2011). These results indicate whether a specific dough formulation will result in a sticky dough (a weak gluten network), or stable dough (strong gluten network). For a stable dough with a strong gluten network, farinograph results indicated increased peak time, a decreased mixing tolerance index (MTI) value and an increased dough stability time, illustrating more protein-protein interactions (Salovaara, 1982a; Letang et al., 1999; Kaur et al., 2011). Both Salovaara (1982a) and Kaur et al. (2011) examined partial NaCl replacement with potassium chloride (KCl), calcium chloride ( $\text{CaCl}_2$ ) and magnesium chloride ( $\text{MgCl}_2$ ). Kaur et al. (2011) reported that 100% replacement of NaCl with KCl and  $\text{CaCl}_2$  caused a significant increase in water absorption showing that 100% replacement of NaCl is not feasible as this could lead to stickier dough because there would be more unabsorbed water available at the dough's surface to participate in adhesive forces. This increased hydration of the gluten proteins would cause a decrease in cohesive forces within the dough. Kaur et al. (2011) also found that with lower levels (~25%) of substitution, water absorption decreased slightly, which is beneficial because more protein-protein interactions occurred so as to create a stronger gluten network and stronger dough through increased cohesive forces. Therefore, lower levels of

replacement may be feasible in decreasing the amount of NaCl used. Salovaara (1982a) found that with the addition of  $\text{CaCl}_2$  and  $\text{MgCl}_2$  peak times were significantly shorter than with 100% NaCl. This is not ideal because longer peak times are desired for the creation of a stronger gluten network to prevent potential over-mixing. However, Salovaara (1982a) found that by replacing NaCl with KCl, no significant changes in peak time were observed. Kaur et al. (2011) found that replacing NaCl with both 25% and 50% KCl led to a slight increase in peak time, which is beneficial for creating a strong gluten network.

Based on these studies, it can be suggested that KCl at a NaCl replacement concentration of ~25-50% would maintain dough rheology while achieving a significant reduction in sodium in the final product. This partial replacement formulation also prevents sticky dough in a similar manner as 100% NaCl by shielding protein charges resulting in protein aggregation so as to create a stronger gluten network with greater cohesive forces. The mechanism is based on the fact that  $\text{K}^+$  is a monovalent ion similar to  $\text{Na}^+$  both chemically and physically. Potassium has been ranked equivalent to  $\text{Na}^+$  in the lyotropic series meaning that they would have similar abilities to cause protein aggregation and have a similar strengthening effect on the gluten network. They are considered stabilizing cations that cause less hydration of the proteins and increased protein structure formation. Although KCl is a possible option for  $\text{Na}^+$  reduction in bakery products, a significant drawback is the metallic off-flavor imparted by this compound. Further research is required to discover an alternative ingredient that will combat both the sticky dough and off-flavor, whilst taking into account all the other factors that may interact to cause sticky dough.

### ***2.7.2 Enzymes as an alternative to salt***

Enzymes have demonstrated the potential to overcome the sticky dough problem; however, studies to date have not been conducted under low sodium conditions. The components of the flour that can be modified by enzymes include: proteins (therefore the gluten network), lipids, pentosans, and starch, which ultimately would modify the dough rheology and impact the dough stickiness (Steffolani et al., 2012). Steffolani et al. (2012) examined a combination of enzymes (glucose oxidase [promotes protein cross-linking],  $\alpha$ -amylase [hydrolyzes starch into  $\alpha$ -dextrins], and xylanase [hydrolyzes water insoluble arabinoxylans into LMW water soluble arabinoxylans]) and their effect on dough stickiness/hardness. Glucose oxidase was found to

decrease dough stickiness with increasing concentrations of enzyme since it led to the formation of a stronger gluten network. In contrast, xylanase was found to increase dough stickiness with increasing concentrations of enzyme as a result of excessive pentosan hydrolysis and thus, higher water absorption. Alpha-amylase however, was found to have little effect on dough stickiness. With an optimum combination of the glucose oxidase, xylanase, and  $\alpha$ -amylase it was found that the effects of each enzyme could be balanced to create dough with low stickiness (Steffolani et al., 2012). Other enzymes (i.e., transglutaminase, glucose oxidase, hexose oxidase, laccase), directly or indirectly, can also be used to enhance the formation of the gluten network by promoting the formation of covalent bonds between polypeptide chains through oxidative cross-linking of SH groups, cross-linking tyrosine residues, or acyl-transfer reactions between amino acid residues (Joye et al., 2009). With low levels of transglutaminase, the dough development time, dough stability, and resistance to extension increased and water absorption, extensibility, and stickiness decreased (Basman et al., 2002; Tseng et al., 2002; Caballero et al., 2005; Joye et al., 2009). Therefore, enzymes with the ability to alter dough properties may be a prospective alternative to salt replacers in the battle against sticky dough.

## **2.8 CONCLUSIONS**

Although the ultimate goal of governmental regulators (e.g., Health Canada) is to increase the health and well-being of its population and to decrease stress on the health care system, sodium reduction policies can create significant techno-functional issues for large scale bakeries. The challenge of decreasing or removing salt from bread formulations, when compared to other processed foods, is not only about the loss of flavour, but also about the loss of dough handling properties and final bread product quality. However, a greater understanding of the stickiness phenomenon is needed in order to help develop strategies to mediate the effect of salt reduction on dough handling.

### **3. CHARACTERIZATION OF FOUR CANADA WESTERN RED SPRING (CWRS) WHEAT FLOURS AND THEIR COMPOSITIONAL EFFECT ON DOUGH FORMATION AND STRENGTH**

#### **3.1 ABSTRACT**

The chemical composition of flours milled from four different CWRS wheat cultivars (Pembina, Roblin, McKenzie and Harvest) were analyzed, and then related to the rheological properties and morphology of their doughs prepared with 2% NaCl. All cultivars showed similar proximate composition and were all of high quality with minimal enzymatic activity/degradation. McKenzie and Harvest cultivars had significantly higher levels of damaged starch (~7.1%) than Pembina and Roblin (~5.7%), which would have an impact on the hydration of the gluten proteins and thus the formation of the gluten network. The amino acid profiles and free thiol content were similar among the flours, however major differences were noted with respect to gluten quality. Both Pembina and Roblin showed a significantly higher gluten index and gluten performance index, as it relates to solvent retention capacity, than both McKenzie and Harvest. However, dough prepared using Pembina flour showed the greatest resistance to extension relative to the dough prepared with Roblin, McKenzie and Harvest cultivars. Pembina was found to have a higher amount of low molecular weight glutenin (insoluble) proteins relative to Roblin, which had higher amounts of high molecular weight glutenin (insoluble) proteins, possibly accounting for the differences in dough strength. McKenzie and Harvest flours were higher in gliadin contents than Pembina and Roblin. All flours formed a unidirectional gluten network within the dough, with the exception of Harvest which was more porous in nature.

### 3.2 INTRODUCTION

Wheat flour is the main ingredient in bread and is comprised of starch (~70-75%), water (~14%), protein (~10-12%), non-starch polysaccharides [arabinoxylans] (~2-3%), and lipids (~2%) (Goesaert et al., 2005). These major and minor components are all relevant in determining flour quality, which plays a vital role in determining functionality and end product uses. For different bakery products wheat quality is defined differently. For breadmaking, good quality flour generally requires high water absorption, good gluten strength and relatively high damaged starch and arabinoxylans (pentosans) (Kweon et al., 2011). A dough with good handling properties is one that is stable and has a strong gluten network. Research indicates that an increased peak time/dough development time, decreased mixing tolerance index value, increased dough stability time, increased resistance to extension, and increased extensibility all indicate a greater amount of protein-protein interactions and thus a stronger gluten network (Hlynka, 1962; Salovaara, 1982a; Linko et al., 1984; Letang et al., 1999; Kaur et al., 2011; Belz et al., 2012). These qualities along with the balance of resistance to extension and extensibility result in a high quality final bread product with good loaf volume and a uniform crumb structure (Barak et al., 2013; Delcour & Hoseney, 2013b). Structurally, in the dough, the main elements are the starch (native and damaged), non-starch polysaccharides, water and air which are all embedded in the water-soluble and insoluble gluten proteins (Jekle & Becker, 2011). Therefore the gluten proteins and starch play a major role in determining dough handling properties.

During the mixing stage of dough formation, the gluten proteins become hydrated, aligned and interconnected due to shear and form a continuous viscoelastic gluten network (Jekle & Becker, 2011). This network is comprised of the glutenins and gliadins, and together they impart dough properties of strength, elasticity, cohesiveness, viscosity, extensibility and contribute to water absorption (Wieser, 2007; Joye et al., 2009). The extent of each characteristic is highly dependent on the quality and ratio of gliadins and glutenins as each serve a different role (Goesaert et al., 2005; Joye et al., 2009). Viscosity and extensibility are imparted to the dough through the gliadins, which are monomeric proteins with molecular weights ranging from 30,000 - 80,000 Da, and thus act as plasticizers within the dough system (Oates, 2001; Goesaert et al., 2005; Joye et al., 2009; Delcour et al., 2012). They are soluble in aqueous alcohol. The glutenins however are a heterogeneous mixture of polymeric proteins with molecular weights ranging from 80,000 - several million Da and form a continuous network

through inter-molecular disulphide bonding which gives the gluten network its elasticity, cohesiveness and strength (Veraverbeke & Delcour, 2002; Joye et al., 2009; Delcour et al., 2012). They are highly insoluble due to their very large size, however, with the use of a reducing agent the disulphide bonds within the glutenin network are broken and result in high molecular weight and low molecular weight glutenin subunits (HMW-GS, LMW-GS) which are soluble in aqueous alcohol (Delcour et al., 2012). There are generally two parameters that define gluten protein quality: the ratio of glutenin to gliadin, and the composition of the glutenins (HMW-GS vs LMW-GS) (Janssen et al., 1996; Goesaert et al., 2005).

The ratio of glutenin to gliadin has a significant effect on the dough handling properties with respects to development time, stability and viscosity; as well as the final bread product with respects to bread loaf volume and crumb texture and structure (Barak et al., 2013). When a wheat cultivar has a lower ratio of glutenins to gliadins it results in dough with decreased resistance to extension and increased extensibility. This is due to the formation of a weaker gluten network because the higher amount of gliadins weaken the interactions between the glutenin chains and can act as chain terminators. However, when there is a higher ratio of glutenins to gliadins it results in increased dough elasticity because of the increase in glutenin polymers present to form the continuous network. This allows for better dough handling properties and an increase in loaf volume as a result of gas expansion (Khatkar et al., 1995; Janssen et al., 1996; Uthayakumaran et al., 2000; Sliwinski et al., 2004). A balance between the dough properties exhibited from the glutenins and the gliadins is necessary for quality bread production.

Differences in the quality of the glutenin fraction arises from the composition (number and position of cysteine residues), secondary structure with respect to non-covalent interactions between glutenin chains, and size distribution (Veraverbeke & Delcour, 2002; Goesaert et al., 2005). The glutenin macropolymer is made up of HMW-GS and LMW-GS. The HMW-GS have molecular weights ranging from ~70,000 - 90,000 Da and the LMW-GS have molecular weights ranging from ~30,000 - 60,000 Da (Oates, 2001; Veraverbeke & Delcour, 2002; Goesaert et al., 2005; Delcour et al., 2012). In the research it is the HMW-GS polymers that are considered the most important for the formation of a continuous gluten network and good dough handling properties as they are believed to form the backbone of the network with the LMW-GS polymers branching off from the main HMW-GS chain (Wieser, 2007). The HMW and LMW-GS have repetitive regions of glutamine, glycine and proline with N and C-terminals which contain the



cysteine amino acids. Through these regions of repetition non-covalent bonds are capable of being formed to serve for increased protein-protein interaction through hydrogen bonding with the high level of glutamine, ionic bonds with the low levels of charged amino acids (between acidic and basic groups) and hydrophobic bonds formed through the interaction of the aromatic amino acids (Hoseney, 1998d; Wieser, 2007). Although there are only a few cysteine amino acids in the ends of the glutenin subunit N and C terminals, they are crucial for forming the disulphide bonds which strengthen the gluten network. All these types of bonds are responsible for the structural and handling properties of the dough (Wieser, 2007).

The starch (both native and damaged) and non-starch polysaccharides (pentosans) also have an effect on dough handling properties as they are capable of absorbing large amounts of water and thus effect gluten protein hydration and the rheological properties of the dough with respect to the viscous properties (Oates, 2001; Goesaert, 2005; Bekes et al., 2006). In the mixing stage, the native starch granules are capable of absorbing 46% of the water and are believed to act as fillers within the continuous gluten network and reinforce the gluten network upon baking (Goesaert et al., 2005). A limited amount of damaged starch is considered desirable for breadmaking as it is able to absorb more water than native starch and it is susceptible to enzymatic hydrolysis, thus it supports the fermentation stage (Goesaert, 2005; Delcour et al., 2010). However, flour that has too much damaged starch is detrimental to the dough functionality as it can negatively impact the handling properties and the final baking performance (Delcour et al., 2010). Petrofsky and Hoseney (1995) found that starch added at different levels but constant gluten levels gave large rheological differences which led them to conclude that starch plays an active role in determining dough rheological characteristics. Therefore, it is important to understand the connection between changes of the microstructure of the gluten network and starch (damaged vs native) as it relates to the macroscopic properties of the dough (Jekle & Becker, 2011).

The overall goal of this research was to characterize the composition of four Canada Western Red Spring (CWRS) wheat flours, and then relate their chemistry to the rheological properties of the dough.

### 3.3 MATERIALS AND METHODS

#### 3.3.1 Materials

Four CWRS flour cultivars (Pembina, Roblin, McKenzie, and Harvest) were kindly provided by the Crop Development Center at the University of Saskatchewan (Saskatoon, SK). The four cultivars were grown in plots at the University of Saskatchewan's Kemen Crop Research Farm under rain-fed conditions on fallowed land. The previous crops in the rotation were canola, pea and barley. All cultivars were seeded May 21, 2013 and each cultivar was grown in a 2.4 m wide by 15.2 m long plot with row spacing of 17.8 cm. The herbicide, fungicide and fertilizer used were: Buctril M (1.0 L/ha) [applied June 11, 2013], Headline (0.4 L/ha) [applied June 28, 2013], and 56.0 kg/ha 23-23-0-10 [applied with seed], respectively. For seed conditioning: 13/64 round holed screen to scalp, 5.5 X ¾ slotted and 12 triangle buckwheat screens to sift were used. The cleaned amounts for each cultivar were: Pembina-40 kg, Roblin-48 kg, McKenzie-42 kg and Harvest-60 kg. Pembina was developed at the Canada Department of Agriculture Research Station (Winnipeg, MB, Canada) by the Rust Area Project Group; it is unlikely to be grown outside of the rust area and is best suited to the Red River Valley (Campbell, 1963). Pembina is a cultivar that has an excellent baking quality, a yield comparable to that of Selkirk grown in the Red River Valley, slightly more rust resistance than Selkirk and has a one day earlier maturation than Selkirk (Campbell, 1963). Roblin is a cultivar for the eastern prairies of Canada that was developed at the Agriculture Canada Research Station (Winnipeg, MB, Canada) it has a similar quality to Marquis, and is a high-protein, early-maturing and rust-resistant cultivar (Campbell & Czarnecki, 1987). McKenzie was developed by the Saskatchewan Wheat Pool (Agricultural Research and Development, Saskatoon, SK, Canada) and has a high grain yield; early maturity; high test weight; high falling number; resistance to stem rust, leaf rust, and common bunt; and has intermediate resistance to Fusarium head blight (Graf et al., 2003). McKenzie is a cultivar suited to all areas of the Canadian Prairies (Graf et al., 2003). Harvest is a cultivar that was developed by Agriculture and Agri-Food Canada (Cereal Research Centre, Winnipeg, MB, Canada) that is suitable to the wheat-producing regions of the Prairies (Fox et al., 2010). Harvest has the qualities of high-yield, high test weight and preharvest sprouting resistance (Fox et al., 2010). Milling of the four selected wheat cultivars was done on a Buhler MLU202 Experimental Mill according to the American

Association of Cereal Chemists International approved method 26-21.02. Prior to milling the grain was tempered to a moisture of 15.5% for ~ 16 hrs.

The following chemicals were purchased from VWR (Mississauga, ON, Canada): 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) [Ellman's reagent], hydrochloric acid (HCl), lactic acid (C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), sodium chloride (NaCl) and sodium dodecyl sulfate (SDS). The following chemicals were purchased from Sigma Aldrich (Oakville, ON, Canada): Ethylenediaminetetraacetic acid (EDTA), rhodamine B, sucrose (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>), Tris-hydrochloride (Tris-HCl), urea (CH<sub>4</sub>N<sub>2</sub>O), Cleland's reagent (dithiothreitol [DTT]) high purity, ribonuclease A from bovine pancreas, trifluoroacetic acid, trizma-base, trizma-HCl and vinyl pyridine. The following chemicals were purchased from Fischer Scientific (Toronto, ON, Canada): acetonitrile HPLC grade and 1-propanol HPLC grade. Nitrogen gas (N<sub>2</sub>) was purchased from Praxair Canada Inc. (Saskatoon, SK, Canada). All chemical reagents were of ACS reagent grade except for SDS which was ultrapure. The water used in this research was produced from a Millipore Milli-Q™ water purification system (Millipore Corp., Milford, MA, USA).

### **3.3.2 Characterization of the flours**

#### *3.3.2.1 AACCI approved flour characterization methods*

The protein, ash, moisture and lipid contents within the flours were determined using the American Association of Cereal Chemists International (AACCI) approved methods 46-30.01, 08-03.01, 44-15.02 and 30-25.01, respectively. Rapid viscoanalysis (RVA) (Std. method 2), the gluten index (GI), the falling number, the damaged starch content and single kernel characterization (SKCS) were also performed according to AACCI methods 76-21.01, 38-12.02, 56-81.03, 76-31.01 and 55-31.01, respectively. Farinograph and mixograph analysis were performed according to AACCI methods 54-21.02 and 54-40.02, respectively. Doughs for mixograph analysis were mixed to a total time of 10 min in order to achieve a full analysis of dough development and breakdown. Measurements were performed in duplicate.

#### *3.3.2.2 Amino acid (AA) profiling*

Amino acid analysis was performed on each flour at POS BioSciences Corp. (Saskatoon, SK, Canada) utilizing acid/heat hydrolysis followed by quantification using chromatographic techniques. In brief, ~20 mg of each flour was weighed into separate 20 x 150 mm screw cap

Pyrex tubes containing 15 mL of 6 N HCl. Each tube was then flushed with N<sub>2</sub> gas. The tubes were then capped and placed into an oven at 110°C for 20 h. After acid hydrolysis, the individual amino acids were quantified by high pressure liquid chromatography using the pico-tag amino acid analysis system (Waters Corporation, Milford, MA, USA) (White et al., 1986; Landry & Delhaye, 1993; AOAC Official Method 985.28, 1995; AOAC Official Method 988.15, 1995).

#### *3.3.2.3 Free thiol content*

Free thiol contents were determined on each flour based on the combined methods of Anderson and Ng (2000), Puppo et al. (2005) and Steffolani et al. (2010). All measurements were performed on flour samples in triplicate. For free thiol content determination, 2 mL Eppendorf tubes were prepared with 1.5 mL reaction buffer [8 M urea, 3 mM EDTA, 1% SDS and 0.2 Tris-HCl at pH 8], 50 µL of Ellman's reagent [8 M urea, 3 mM EDTA, 1% SDS, 0.2 Tris-HCl and 10 mM DTNB at pH 8]. Then ~30 mg of flour was added to each tube and vortexed for 30 s on speed 8 (VWR analog vortex mixer 120V, Mississauga, ON, Canada). The samples were then placed on an agitation shaker (VWR® Standard Orbital Shaker, Model 3500, Mississauga, ON, Canada) at speed 4 for 1 h in the dark. Samples were then placed in a microcentrifuge (Eppendorf® Microcentrifuges, 5424/5424R, Mississauga, ON, Canada) for 10 min at 13,600 x g at room temperature (21-23°C). The absorbance of the supernatants was read at 412 nm using a UV-VIS spectrophotometer (Genesys 10uv Scanning Thermo Scientific, Waltham, MA, USA). An extinction coefficient of 13,600 M<sup>-1</sup>cm<sup>-1</sup> and a microcuvette with a path length of 1 cm was used. A blank was prepared for each flour (~ 30 mg) and contained 1.55 mL of the reaction buffer (i.e., 1.55 mL of reaction buffer to get a total volume of 1.55 mL instead of 1.5 mL and 50 µL of colour reagent).

#### *3.3.2.4 Solvent retention capacity (SRC)*

A solvent retention test was carried out on each of the four flours (Pembina, Roblin, McKenzie, and Harvest) utilizing the AACCI method 56-11.02 (2009) with a slight modification, to establish the gluten protein characteristics, levels of damaged starch, sucrose and pentosan components. Four solvents were prepared and used on each flour: (1) deionized and distilled water (DDH<sub>2</sub>O) (associated with all water absorbing components in flour); (2) 50% sucrose in DDH<sub>2</sub>O (w/w) (associated with pentosan components); (3) 5% sodium carbonate in

DDH<sub>2</sub>O (w/w) (associated with levels of damaged starch); and (4) 5% lactic acid in DDH<sub>2</sub>O (w/w) (associated with glutenin proteins). First, the weight of a 50 mL conical bottom polypropylene centrifuge tube (with lid) was recorded and then 5 g of a flour sample was weighed into the tube to enable for mixing with the solvent. Then 20 g of one of the solvents (i.e., solvent 1, 2, 3 or 4) was added to the tube and vortexed at setting at speed 10 (VWR analog vortex mixer 120V, Mississauga, ON, Canada) for 5 s at 5, 10, 15, and 20 min. The sample was then centrifuged for 15 min at 1000 x g (VWR Clinical 200, Mississauga, ON, Canada). Then the tube was inverted and drained for 10 min and the remaining residue weighed. These steps were repeated with each of the solvents on the same flour sample and then repeated for the 3 other flour samples. Measurements were performed on triplicate flour samples. To calculate the percent SRC, equation 3.1 below was used. Then from the calculated SRC for each component the gluten performance index (GPI) was calculated using the formula in equation 3.2 (Kweon et al., 2011).

$$\% SRC = \left[ \frac{\text{gel weight}}{\text{flour weight}} - 1 \right] * \left[ \frac{86}{100 - \% \text{ flour moisture}} \right] * 100 \quad (\text{Eq. 3.1})$$

$$GPI = \frac{\text{Lactic acid SRC}}{(\text{sodium carbonate SRC} + \text{sucrose SRC})} \quad (\text{Eq. 3.2})$$

The GPI, which is a new predictive parameter, is used to describe the overall performance of glutenin while in the network system of the other wheat flour polymers (Kweon et al., 2011; Hammed et al., 2015).

### **3.3.3 Gluten protein fractionation using reversed-phase high-performance liquid chromatography (RP-HPLC)**

#### *3.3.3.1 Sample preparation*

Gluten protein fractionation was carried out based on a modified version of Fu and Sapirstein (1996), Sapirstein and Fu (1998), and Naeem and Sapirstein (2007). A flow chart summarizing the extraction procedure is given in Figure 3.1. For each flour cultivar, 50 mg was weighed into 2 mL microcentrifuge tubes in triplicate. The flour was then extracted for 15 min with 1 mL of 50% (v/v) 1-propanol with intermittent vortexing (~ every 5 min) (Thermolyne, Maxi Mix II Type 37600 Mixer, TX, USA). Samples were then centrifuged (Thermo Scientific,

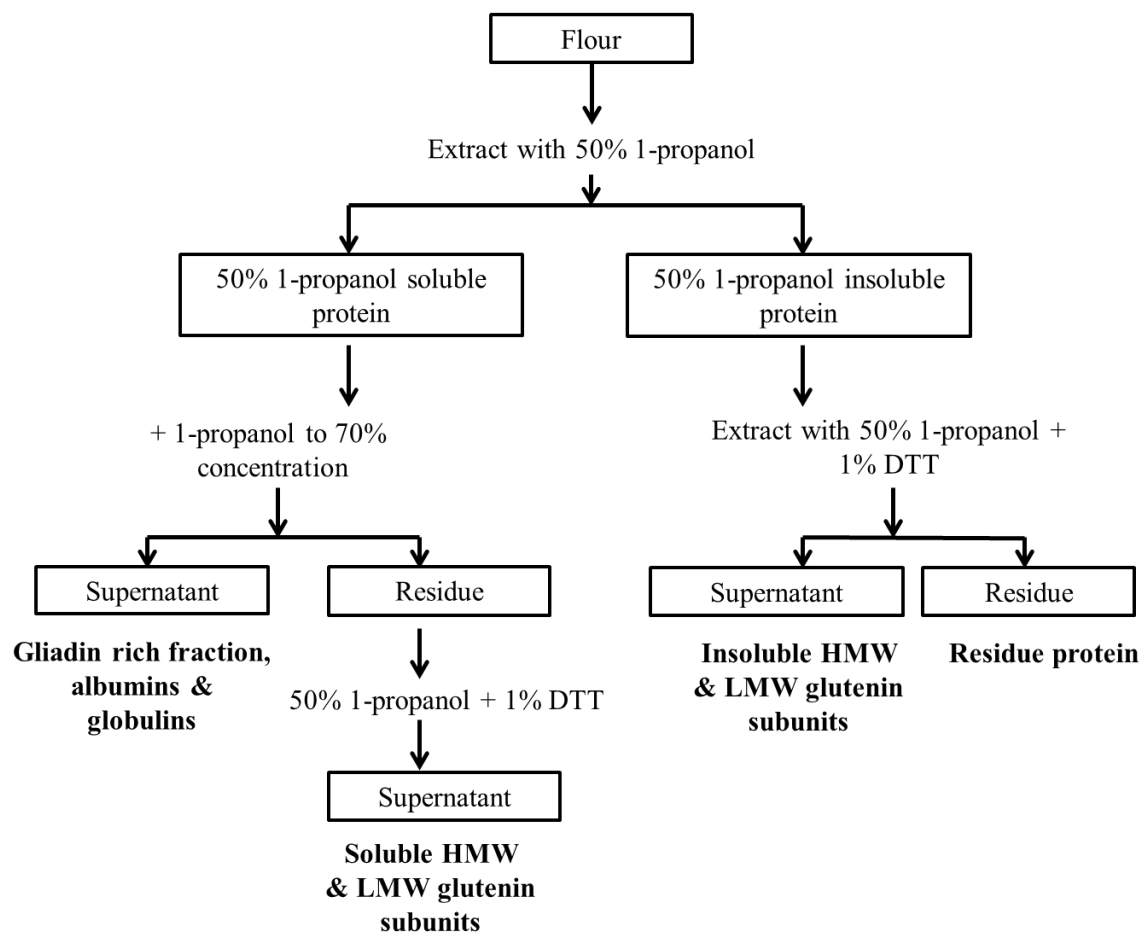


Figure 3.1 Gluten protein preparation procedure for fractionation by reverse-phase high-performance liquid chromatography. Adapted from Fu and Sapirstein (1998).

Sorvall Legend Micro 21R Centrifuge, ON, Canada) (3 min, 15,000 x g) and the resulting supernatant was decanted into a 2 mL microcentrifuge tube. This extraction was then repeated a second time with pellet disruption, using a small glass rod, before extraction to increase extraction efficiency. The supernatant was then pooled with the first supernatant, and represents the 50% 1-propanol soluble protein fraction in Figure 3.1. The pellet was then rinsed with ~ 500 µL of 50% 1-propanol, the liquid drained and then the microcentrifuge tube was inverted over a paper towel to allow the residue to air dry (representing 50% 1-propanol insoluble fraction in Figure 3.1).

One hundred microliters of a reducing solution (0.08 M Tris-HCl pH 7.5 buffer containing 50% 1-propanol and 1% dithiothreitol [DTT]) was added to the 50% 1-propanol insoluble protein fraction and the residue was then disrupted using a small glass rod. The sample was then heated in a heating block (55°C, 30 min) (VWR Scientific, Heat Block, Canada, ON) and vortexed intermittently (~every 10 min). Then 100 µL of an alkylating solution (0.08 M Tris-HCl pH 7.5 containing 50% 1-propanol and 4% (v/v) 4-vinylpyridine) was added, and then the sample was placed in the heating block again (55°C, 30 min) and vortexed intermittently (~every 10 min). The sample was then centrifuged (Thermo Scientific, Sorvall Legend Micro 21R Centrifuge, ON, Canada) for 5 min at 15,000 x g and the supernatant was decanted and syringe filtered (0.45 µm Millex HV) into HPLC vials. This fraction contains the insoluble HMW and LMW glutenin subunits (Figure 3.1). The residue protein remaining after this extraction was discarded.

The concentration of 1-propanol for the 50% 1-propanol soluble protein fraction was increased to 70% (v/v), vortexed, and then allowed to sit for 1 hr. The sample was centrifuged for 10 min at 20,000 x g (Thermo Scientific, Sorvall Legend Micro 21R Centrifuge, ON, Canada). The resulting supernatant (containing the gliadin rich fraction) was decanted as waste and the residue in the microcentrifuge tube was inverted on paper towel and allowed to dry. Using the residue obtained from the 70% 1-propanol extraction, the soluble HMW and LMW glutenin subunits were obtained using the same process described above for 50% 1-propanol insoluble HMW and LMW glutenin subunits starting with the reducing solution (Figure 3.1), alkylating solution, centrifugation and syringe filtration into HPLC vials.

#### *3.3.3.2 Reverse-phase high-performance liquid chromatography*

Reverse-phase high-performance liquid chromatography was carried out according to Fu and Sapirstein (1996) and, Naeem and Sapirstein (2007). Supernatant fractions containing the insoluble and the soluble glutenins were analyzed using an Agilent HPLC 1100 system containing a binary solvent delivery system, auto-sampler, online vacuum degasser and diode array detector with a 6 mm path length, and 1.7 mL flow cell. Depending on whether stainless steel or not, tubing that was critical from the injector inlet to the column inlet, as well as from the column outlet to the flow cell, had an internal diameter of 0.12 and 0.13 mm, respectively. The small tubing diameter was utilized to minimize peak elution times and maximize peak resolution.

Eluents utilized were solvent A, comprised of DDH<sub>2</sub>O and 0.1% (v/v) trifluoroacetic acid (TFA), and solvent B comprised of acetonitrile and 0.1% (v/v) TFA. The elution of glutenin subunits was monitored at a wavelength of 206 nm. Injection volume was 1 µL for the 50% 1-propanol insoluble fraction and 3 µL for the 70% 1-propanol insoluble fraction. Glutenin subunit separation was obtained with a flow rate of 0.2 mL/min at 60°C with an elution gradient as follows (time, % solvent B): 0 min, 23% B; 3 min, 23% B; 54 min, 44% B; 55 min 23% B, and a post run time of 10 min. For integration and quantitative analysis of the resulting chromatographs, Agilent ChemStation software (version 10.01) was used with a 0.05 min peak width response time. The column utilized was a Zorbax 300 SB-C8 (Rockland Technologies, Inc., Newport, DE) with a 300 Å pore size, 3.5 µm particle size, 2.1 mm diameter and a length of 100 mm. The quantification of the integrated peak areas was done by using a pure protein standard (ribonuclease A from bovine pancreas). Utilizing the conditions described above, a dilution series of ribonuclease A in 0.08 M Tris-HCl pH 7.5 buffer with 50% 1-propanol was run. This was then used to determine the protein contents corresponding to milli-Absorbance Units.

### **3.3.4 Dough extensibility**

All dough was prepared using a 10 g mixograph (TMCO National Mfg., Lincoln, NE) utilizing a formulation comprised of flour (weight on a 14% moisture basis), water (weight based on farinograph absorption results) and NaCl salt (2% on flour weight basis), in triplicate. All doughs were mixed until just after reaching peak time. Dough extensibility was measured utilizing a stable microsystems (SMS) TA.XTplus Texture profile analyzer equipped with a 5 kg load cell and an SMS/Kieffer rig with modifications to the procedure (Stable Micro Systems, 2005). Settings for the test were as follows: pretest speed of 2.0 mm/s, test speed of 3.3 mm/s, posttest speed of 10.0 mm/s, distance of 100.0 mm and a trigger force of 5.0 g. The dough was not rested before loading into the oiled form. Once the dough was in the dough clamp the dough was rested for 10 min at room temperature (21-23°C). After the resting period, the dough strips were then loaded onto the platform and measurements were taken. The measurements taken were dough resistance to extension (N), which is the force at the maximum height of the curve and dough extensibility (mm) which is the length of the curve upon dough breaking.



### **3.3.5 Confocal laser scanning microscopy (CLSM)**

CLSM was utilized to visualize the gluten network formation of the four different wheat flours with 2% NaCl and was based off of the method by Jekle and Becker (2011). The doughs were prepared as described above. The fluorescent dye rhodamine B was prepared at a concentration of 0.001% (w/v) in DDH<sub>2</sub>O and was then utilized as the stock water addition to each dough formulation to ensure that the dye was distributed homogeneously throughout the dough during the mixing process. A small sample was then taken from the dough with two plastic spatulas to try to prevent stretching of the dough, the sample was about 4-5 mm in diameter. The dough sample was then loaded on the slide and flattened with the cover slip by placing the cover slip overtop and then turning the slide over and lightly pushing it down on the table. All samples were loaded this way. The dough was analyzed by a Nikon C2 CLSM microscope (Nikon, Mississauga, ON, Canada) using 20x Plan Fluor (numerical aperture 0.75, Nikon) objective lens. The excitation and emission wavelengths were 543 nm and 625 nm (rhodamine B), respectively. A total of 10 images with 512 x 512  $\mu\text{m}$  pixel resolution were taken for each dough sample from different positions on the *xy*-axis. Each dough sample was analyzed in duplicate. Six images of each dough were accessed for particle count, total particle area, average particle size, circularity and fractal dimension using Image J software (National Institutes of Health, Bethesda, MD, USA) based on Jekle and Becker (2011).

### **3.3.6 Statistical analysis**

A one way analysis of variance (ANOVA) using a Scheffe Post-hoc test was used to show statistical differences for flour cultivars for parameters used to characterize the flours and for dough extensibility and confocal image analysis experiments. The statistical analysis program SPSS (Armonk, NY, USA) was utilized.

## **3.4 RESULTS AND DISCUSSION**

### **3.4.1 Flour characterization**

Analysis of a wide range of standard flour properties are given in Table 3.1. The CWRS flours tested ranged in protein levels from 14.7% to 16.2% (d.b.), which were similar in magnitude to other CWRS (Edwards et al., 2012) and hard wheat classes (Delcour et al., 2012). In the present study, Roblin flour contained the highest protein content (~16.2% d.b.), followed

Table 3.1 Flour properties for four CWRS wheat cultivars Pembina, Roblin, McKenzie and Harvest. Data represents the mean  $\pm$  one standard deviation (n = 2).

Characteristics <sup>1</sup>	Flours <sup>2</sup>			
	Pembina	Roblin	McKenzie	Harvest
<b>Proximate analysis</b>				
a. Protein (% on 14% w.b.)	12.6 $\pm$ 0.0 <sup>a</sup>	13.9 $\pm$ 0.0 <sup>c</sup>	13.0 $\pm$ 0.0 <sup>b</sup>	13.0 $\pm$ 0.1 <sup>b</sup>
b. Protein (% d.b.)	14.7 $\pm$ 0.0 <sup>a</sup>	16.2 $\pm$ 0.0 <sup>c</sup>	15.1 $\pm$ 0.0 <sup>b</sup>	15.1 $\pm$ 0.1 <sup>b</sup>
c. Lipid (% d.b.)	1.13 $\pm$ 0.01 <sup>b</sup>	1.08 $\pm$ 0.00 <sup>b</sup>	0.93 $\pm$ 0.03 <sup>a</sup>	1.03 $\pm$ 0.04 <sup>ab</sup>
d. Ash (% d.b.)	0.52 $\pm$ 0.00 <sup>b</sup>	0.46 $\pm$ 0.01 <sup>a</sup>	0.53 $\pm$ 0.01 <sup>b</sup>	0.53 $\pm$ 0.0 <sup>b</sup>
<b>Falling number (s)</b>	475 $\pm$ 16 <sup>a</sup>	470 $\pm$ 17 <sup>a</sup>	483 $\pm$ 17 <sup>a</sup>	486 $\pm$ 21 <sup>a</sup>
<b>SKCS - HI</b>	67.43 $\pm$ 0.67 <sup>a</sup>	65.59 $\pm$ 1.34 <sup>a</sup>	77.82 $\pm$ 0.55 <sup>c</sup>	73.53 $\pm$ 0.97 <sup>b</sup>
<b>Damaged starch (%)</b>	5.97 $\pm$ 0.26 <sup>a</sup>	5.45 $\pm$ 0.10 <sup>a</sup>	7.06 $\pm$ 0.20 <sup>b</sup>	7.06 $\pm$ 0.22 <sup>b</sup>
<b>Gluten Index (%)</b>	84.3 $\pm$ 2.9 <sup>c</sup>	88.1 $\pm$ 1.5 <sup>c</sup>	66.3 $\pm$ 1.8 <sup>b</sup>	48.6 $\pm$ 0.8 <sup>a</sup>
a. Wet Gluten (%)	36.0 $\pm$ 0.6 <sup>a</sup>	42.4 $\pm$ 0.1 <sup>c</sup>	40.1 $\pm$ 0.4 <sup>b</sup>	42.1 $\pm$ 0.3 <sup>c</sup>
b. Dry Gluten (%)	12.2 $\pm$ 0.3 <sup>a</sup>	14.8 $\pm$ 0.1 <sup>c</sup>	13.7 $\pm$ 0.3 <sup>b</sup>	13.8 $\pm$ 0.1 <sup>b</sup>
<b>Rapid visco-analysis (RVU)</b>				
a. Peak viscosity	123.3 $\pm$ 2.1 <sup>a</sup>	141.9 $\pm$ 0.3 <sup>b</sup>	129.3 $\pm$ 0.8 <sup>a</sup>	140.1 $\pm$ 1.6 <sup>b</sup>
b. Breakdown viscosity	35.3 $\pm$ 2.5 <sup>a</sup>	42.6 $\pm$ 0.4 <sup>b</sup>	36.0 $\pm$ 0.4 <sup>a</sup>	36.4 $\pm$ 0.1 <sup>a</sup>
c. Trough viscosity	87.0 $\pm$ 1.0 <sup>a</sup>	99.3 $\pm$ 0.1 <sup>c</sup>	93.3 $\pm$ 0.5 <sup>b</sup>	103.7 $\pm$ 1.5 <sup>d</sup>
d. Setback viscosity	102.3 $\pm$ 1.5 <sup>a</sup>	108.4 $\pm$ 0.1 <sup>b</sup>	108.5 $\pm$ 1.2 <sup>b</sup>	119.6 $\pm$ 0.8 <sup>c</sup>
e. Final viscosity	189.3 $\pm$ 2.5 <sup>a</sup>	207.6 $\pm$ 0.2 <sup>b</sup>	201.8 $\pm$ 1.7 <sup>b</sup>	223.3 $\pm$ 0.8 <sup>c</sup>

<sup>1</sup>Abbreviations: wet basis (w.b.); dry basis (d.b.); single kernel characterization system (SKCS); hardness index (HI); and rapid viscoanalyzer units (RVU).

<sup>2</sup>Different letters represent significantly different values (p<0.05) within a row.

by McKenzie and Harvest (~15.1% d.b.), which were similar, and then Pembina (14.7% d.b.). Pembina had the lowest protein among the cultivars ( $p < 0.01$ ). The lipid contents in all flours ranged between 0.93% (d.b.) for McKenzie to ~1.1% (d.b.) for Roblin/Pembina, with Harvest more mid-range (1.0%, d.b.) (Table 3.1). Values were also within a similar range of others found in the literature, which ranged between ~1.1 and 2.0% (d.b.) (Goesaert et al., 2005; Mak, 2009; Delcour & Hosney, 2013a). Ash levels found in Pembina, McKenzie and Harvest flours were found to be similar (~0.53%, d.b.) ( $p < 0.05$ ), and significantly higher than that of Roblin (0.46% d.b.) ( $p < 0.05$ ). Similar values have been reported in the literature of other CWRS wheat flours by Edwards et al. (2012) and Zghal et al. (2001).

Falling number gives an indication of the degree of enzymatic activity occurring within the wheat; where a high falling number ( $> 300$  s) indicates minimal activity, whereas values  $< 250$  s indicates extensive activity and possible sprout damage to the flour (Wheat Marketing Centre, 2008). In the present study, all flours were found to have similar falling numbers of ~479 s indicating that the flours were of high quality with minimal enzymatic activity or degradation ( $p > 0.05$ ) (Table 3.1). When considering the single kernel characterization system, it can be observed that the two stronger wheat cultivars, Pembina and Roblin have similar hardness indices of ~66 ( $p < 0.05$ ), which were significantly lower than Harvest (~74) and McKenzie (~78) ( $p < 0.05$ ) (Table 3.1). The higher the hardness index is, the harder the kernels are. Damaged starch levels were found to be higher in the cultivars having the harder kernels relative to those with lower hardness indices. For instance, McKenzie/Harvest had significantly greater levels of damaged starch (~7.1%) than that of Pembina/Roblin (~5.7%) ( $p > 0.05$ ) (Table 3.1). Using the roller mill, it would take more force or a greater number of passes through the roller mill to crush the harder kernels which would result in a greater amount of damaged starch.

The gluten index is an indicator of the relative gluten strength of the flour; it is the ratio of wet gluten remaining on the sieve (after centrifuging) to the total wet gluten (AACC International, 2000). The index for Pembina/Roblin flours (~86%) were significantly greater than that of McKenzie (~66%) and Harvest (~47%) ( $p < 0.05$ ) (Table 3.1) indicating that Pembina/Roblin have much stronger gluten networks. Wet gluten gives an indication of the water binding capabilities of the gluten, with Roblin/Harvest having similar values (~42%), which were significantly greater than both McKenzie (~40%) and Pembina (~36%) flours ( $p < 0.05$ ) (Table 3.1). Dry gluten content tends to correlate to protein content of the flour, the

higher the protein content of a flour generally means a higher dry gluten content. It can be seen that the dry gluten content follows the trend for the total protein levels with each flour (Table 3.1); with Roblin having the highest (16.2% d.b.), followed by McKenzie/Harvest (15.1% d.b.) and then Pembina (14.7% d.b.).

In examining the starch properties of the different flours using a rapid visco-analyzer no evidence of sprout damage as the result of high enzymatic activity was observed, which could lead to poor dough handling (Wheat Marketing Centre, 2008). Flours that have sprout damage tend to have low peak viscosities within their curves. All flours studied appeared to be of high quality. Peak viscosity for Pembina/McKenzie (~125 RVU) were found to be significantly lower than that of Roblin/Harvest (~141 RVU) ( $p < 0.05$ ) (Table 3.1). The breakdown viscosity was found to be similar for Pembina, McKenzie and Harvest (~36 RVU), which was significantly lower than that of Roblin (~43 RVU) ( $p < 0.05$ ) (Table 3.1). Trough viscosity found to be significantly different for all flours, where viscosity increased from ~87 RVU (Pembina), to ~93 RVU (McKenzie), to ~99 RVU (Roblin) and to ~104 RVU (Harvest) ( $p < 0.05$ ) (Table 3.1). Set back viscosity found to be lowest for Pembina (~102 RVU), and then increased to ~108 RVU for Roblin/McKenzie, and then again to ~120 RVU for Harvest ( $p < 0.05$ ) (Table 3.1). The final viscosity was found to be lowest for Pembina (~189 RVU), and then increased to ~204 RVU for Roblin/McKenzie, and then again to ~223 RVU for Harvest ( $p < 0.05$ ) (Table 3.1).

#### *3.4.1.1 Amino acid profiles & free thiol content*

The amino acid (AA) profile of each flour was examined and given in Table 3.2. Based on the total AAs, Pembina contained the lowest amount AAs (~15.9 %), followed by McKenzie/Harvest (~16.5%) and then Roblin (~18.2%) (Table 3.2). Results follow a similar trend as those shown in Table 3.1 determined by the Leco combustion method. Reactive sites on the various AAs can have a big impact on the nature of interactions within the dough, such as disulphide, hydrophobic, hydrogen, and ionic bonding. For instance, an amino acid primarily involved in covalent bonding is cysteine, whereas polar/charged AAs (e.g., glutamine/glutamic acid) partake in hydrogen and ionic bonding, and the charged AA can be shielded by salts present within the formulations. Furthermore, bulky aromatic side groups from tryptophan, tyrosine and phenylalanine lead to hydrophobic interactions to help form the gluten network. AA profiles for all flours in the present study were similar in magnitude to other wheat flours found

Table 3.2 Amino acid (AA) profiles of flours from CWRS wheat cultivars Pembina, Roblin, McKenzie and Harvest. Data represent the actual percent AA concentration within flour; whereas numbers within the brackets have been normalized to 100% in order to compare levels in-between cultivars.

Amino Acid (%)	Flours			
	Pembina	Roblin	McKenzie	Harvest
Glycine	0.53 (3.3)	0.61 (3.3)	0.53 (3.2)	0.54 (3.3)
Alanine	0.50 (3.2)	0.55 (3.0)	0.51 (3.1)	0.52 (3.1)
Serine	0.70 (4.4)	0.81 (4.4)	0.72 (4.4)	0.71 (4.3)
Threonine	0.43 (2.7)	0.48 (2.6)	0.44 (2.7)	0.42 (2.5)
Cysteine	0.33 (2.1)	0.35 (1.9)	0.33 (2.0)	0.32 (1.9)
Aspartic acid (+ Asparagine)	0.62 (3.9)	0.71 (3.9)	0.65 (3.9)	0.65 (3.9)
Glutamic acid (+ Glutamine)	5.44 (34.3)	6.54 (35.9)	5.76 (35.0)	5.75 (34.8)
Histidine	0.47 (3.0)	0.55 (3.0)	0.49 (3.0)	0.52 (3.1)
Arginine	0.60 (3.8)	0.71 (3.9)	0.62 (3.8)	0.60 (3.6)
Lysine	0.36 (2.3)	0.33 (1.8)	0.34 (2.1)	0.36 (2.2)
Valine	0.66 (4.2)	0.70 (3.8)	0.67 (4.1)	0.68 (4.1)
Leucine	1.15 (7.3)	1.20 (6.6)	1.15 (7.0)	1.19 (7.2)
Isoleucine	0.63 (4.0)	0.65 (3.6)	0.63 (3.8)	0.65 (3.9)
Methionine	0.25 (1.6)	0.26 (1.4)	0.26 (1.6)	0.24 (1.5)
Proline	1.85 (11.7)	2.18 (12.0)	1.98 (12.0)	1.95 (11.8)
Phenylalanine	0.89 (5.6)	0.94 (5.2)	0.91 (5.5)	0.94 (5.7)
Tyrosine	0.30 (1.9)	0.48 (2.6)	0.34 (2.1)	0.32 (1.9)
Tryptophan	0.15 (0.9)	0.16 (0.9)	0.15 (0.9)	0.15 (0.9)
<b>Total</b>	<b>15.86 (100)</b>	<b>18.21 (100)</b>	<b>16.48 (100)</b>	<b>16.51(100)</b>

in the literature, with slight differences based on flour cultivar and growing conditions/environment the crops experienced (Cornell, 2012; Delcour & Hoseney, 2013a). Gluten proteins are known for being rich in glutamine, proline and hydrophobic AAs (Delcour & Hoseney, 2013a). In Table 3.2, glutamic acid (+ Glutamine), proline and hydrophobic AAs accounted for ~35%, ~12% and ~37% of the total AAs, respectively regardless of the flour cultivar, which is typical for wheat flours.

Cysteine residues were shown to represent ~1.9-2.1% of the total AAs, regardless of the flour (Table 3.2). These values for total cysteine (based on the total AAs) are similar to those found in literature for wheat flours ~1.9-2.5% (Cornell, 2012; Delcour & Hoseney, 2013a). Cysteine AA residues are extremely important in the development of the gluten network as they participate in covalent disulphide bonding. An analysis of variance found the effect of flour cultivar on the free thiol content not to be significant ( $p>0.05$ ). The CWRS flours were found to have an average free thiol content of 1.00  $\mu\text{mol/g}$  flour d.b. Specifically, Pembina, Roblin, McKenzie and Harvest had values of  $0.97 \pm 0.10$ ,  $1.09 \pm 0.08$ ,  $0.92 \pm 0.05$  and  $1.04 \pm 0.08$   $\mu\text{mol/g}$  flour d.b, respectively. These values for free thiol content are also similar to those found in literature. For instance, Steffolani et al. (2010) reported a value of 0.53  $\mu\text{mol/g}$  flour d.b. (based on flour with 10.9% protein), Puppo et al. (2005) found values of 1.42 and 2.43  $\mu\text{mol/g}$  flour d.b. (based on flours with 11.7% and 10.9% protein, respectively) and, Anderson and Ng (2000) found values of 1.05  $\mu\text{mol/g}$  flour d.b. (based on a flour with 9% protein).

#### *3.4.1.2 Solvent retention capacity of flour components*

Solvent retention capacity (SRC) measures the compatibility of three flour components (gluten, damaged starch and pentosans) in different solvents to give a prediction of each components' contribution to the flour's overall quality (with respects to the level of water absorption, gluten strength, damaged starch level and level of pentosans) and finished product quality (for breads, cookies, crackers, cakes, and noodles as each of these products require different flour functionality) (Kweon et al., 2011; Švec et al., 2012). Empirical rheological and baking tests measure the combined contributions of these flour components to flour functionality/ quality whereas, SRC enables for a measurement of the individual functional flour components contribution separately (Kweon et al., 2011). For bread production a good quality flour generally requires a high water absorption, good gluten strength and relatively high

damaged starch and arabinoxylans (pentosans), whereas for cookie production a good quality flour generally requires a low water absorption, very little gluten strength, and a low amount of damaged starch and arabinoxylans (Kweon et al., 2011). In Table 3.3, the SRC of flours within water (*associated with contributions from all flour components*) indicated that McKenzie retained the highest amount of water (~78%) ( $p < 0.05$ ), followed by Roblin (~76%), and then Harvest (~74%) and Pembina (~72%), which were similar in magnitude ( $p > 0.05$ ) (Table 3.3). Contributions to water retention relating to pentosans appeared to be greatest for Roblin and Pembina flours ( $p < 0.05$ ), followed by McKenzie and Harvest flours which were similar ( $p > 0.05$ ). This may give an indication that the level of pentosans within these flour cultivars may not be responsible for dough stickiness within reduced sodium dough formulations given that the two stronger cultivars that produce non-sticky doughs have higher water retentions as a result of the pentosans. However, further testing would be necessary to rule out the contributions of the pentosans. Contributions associated with the gluten proteins was significantly greater in flours from Roblin ( $p < 0.05$ ), followed by Pembina ( $p < 0.05$ ), and then McKenzie and Harvest which were similar ( $p > 0.05$ ) which may reflect the trend in total protein levels within McKenzie and Harvest (Table 3.1). The gluten performance index, predicting the overall performance of glutenin in the presence of the network of other flour polymers, shows more powerful differences between the flour cultivars with Roblin having the highest GPI ( $p < 0.01$ ) followed by Pembina ( $p < 0.01$ ) with McKenzie and Harvest being similar but having the lowest GPI ( $p < 0.001$ ). And finally, the contributions to water retention from damaged starch within the flours was greatest for McKenzie, followed by Pembina, Harvest and then Roblin ( $p < 0.05$ ). McKenzie also had the highest damaged starch level in Table 3.1. It was reported by Hammed et al. (2015) that the sodium carbonate SRC correlated well with damaged starch levels. However, in the current study Pembina showed a higher sodium carbonate SRC than Harvest, despite exhibiting a lower level of starch damage. This finding could be impacted by the vortexing process used in this analysis, which could contribute to slightly different water uptake by damaged starch as compared to the mixing action of dough. Findings for SRC values were similar in magnitude to Hammed et al. (2015) who examined a number of hard red spring wheat flours.

Table 3.3 Solvent retention capacity of flours from CWRs wheat cultivars Pembina, Roblin, McKenzie and Harvest. Data represent the mean  $\pm$  one standard deviation (n = 3).

Flour	Solvent retention capacity, %				
	50% Sucrose (Pentosans)	5% Lactic acid (Gluten proteins)	5% Sodium carbonate (Damage starch)	DDH <sub>2</sub> O (all components)	Gluten performance index
Pembina	128.20 $\pm$ 1.90 <sup>bc</sup>	142.61 $\pm$ 1.02 <sup>b</sup>	95.66 $\pm$ 0.24 <sup>c</sup>	72.28 $\pm$ 0.77 <sup>a</sup>	0.64 $\pm$ 0.01 <sup>b</sup>
Roblin	130.91 $\pm$ 3.34 <sup>c</sup>	151.71 $\pm$ 2.37 <sup>c</sup>	91.19 $\pm$ 0.15 <sup>a</sup>	75.96 $\pm$ 1.26 <sup>bc</sup>	0.68 $\pm$ 0.01 <sup>c</sup>
McKenzie	123.71 $\pm$ 1.84 <sup>ab</sup>	127.28 $\pm$ 1.90 <sup>a</sup>	99.61 $\pm$ 0.23 <sup>d</sup>	77.96 $\pm$ 1.35 <sup>c</sup>	0.57 $\pm$ 0.01 <sup>a</sup>
Harvest	120.03 $\pm$ 0.79 <sup>a</sup>	123.27 $\pm$ 0.14 <sup>a</sup>	94.35 $\pm$ 0.22 <sup>b</sup>	73.68 $\pm$ 0.24 <sup>ab</sup>	0.58 $\pm$ 0.00 <sup>a</sup>
Target ranges*	105-115	>140	80-90	65-70	0.75

Different letters in the same column are significantly different (p<0.05). \*Targeted ranges given were provided from [www.uswheat.org](http://www.uswheat.org).



#### 3.4.1.3 *Gluten protein fractionation*

Protein composition plays an important role in determining wheat quality for dough handling and baking performance. The presence or absence of certain HMW glutenin subunits, LMW glutenin subunits, and gliadins are determinants in protein quality (Suchy et al., 2003), along with the ratio of the glutenins to gliadins (Southan & MacRitchie, 1999). In Figure 3.2, the insoluble (A) and soluble (B) gluten fractions are given, along with their ratio (C) to give an indication of gluten strength. The insoluble glutenin proteins have a strengthening effect on the gluten network as the HMW and LMW glutenin subunits form disulphide bonds to create a more continuous elastic network. Pembina and Roblin, which are known to be strong dough producing flours, have a greater amount of total glutenins (~29%) when compared to McKenzie and Harvest ( $p<0.05$ ) (~25%) which are the weaker dough producing flours.

In Figure 3.2A, it can be seen that the HMW insoluble glutenin fraction was similar between Pembina and Harvest cultivars despite forming strong and weak doughs, respectively. Barak et al. (2013) examined the protein composition in multiple flour cultivars to show no differences within the total HMW insoluble glutenin levels, despite differences in baking performance. The authors attributed the latter to differences in the composition of subunits within the HMW glutenin fraction. For instance, 2\*, 5+10 and 7+9 HMW subunits impart increased dough development and stability, whereas subunits containing N, 2+12 and 20 result in a weakening effect on the dough. Suchy et al. (2003) found something similar where there were significantly different dough properties among cultivars, despite having similar levels of the total insoluble HMW glutenin fraction. The authors attributed the differences observed in the dough properties to differences in LMW glutenin and gliadin composition. In the present study, it is believed that differences in dough properties between the cultivars is attributed to differences in subunit composition within the HMW insoluble glutenin fraction, and also differences in the levels of LMW glutenin and gliadin fractions. For instance, LMW insoluble glutenins level in Pembina was higher (20%) than Harvest (~17%) ( $p<0.05$ ) (Figure 3.2A), and the total gliadins in Pembina were lower (~63%) than Harvest (~66%) (Figure 3.2B). It could possibly be the insoluble LMW glutenin subunits that play a larger role than originally hypothesized in maintaining the gluten network formation and strength in reduced sodium environments

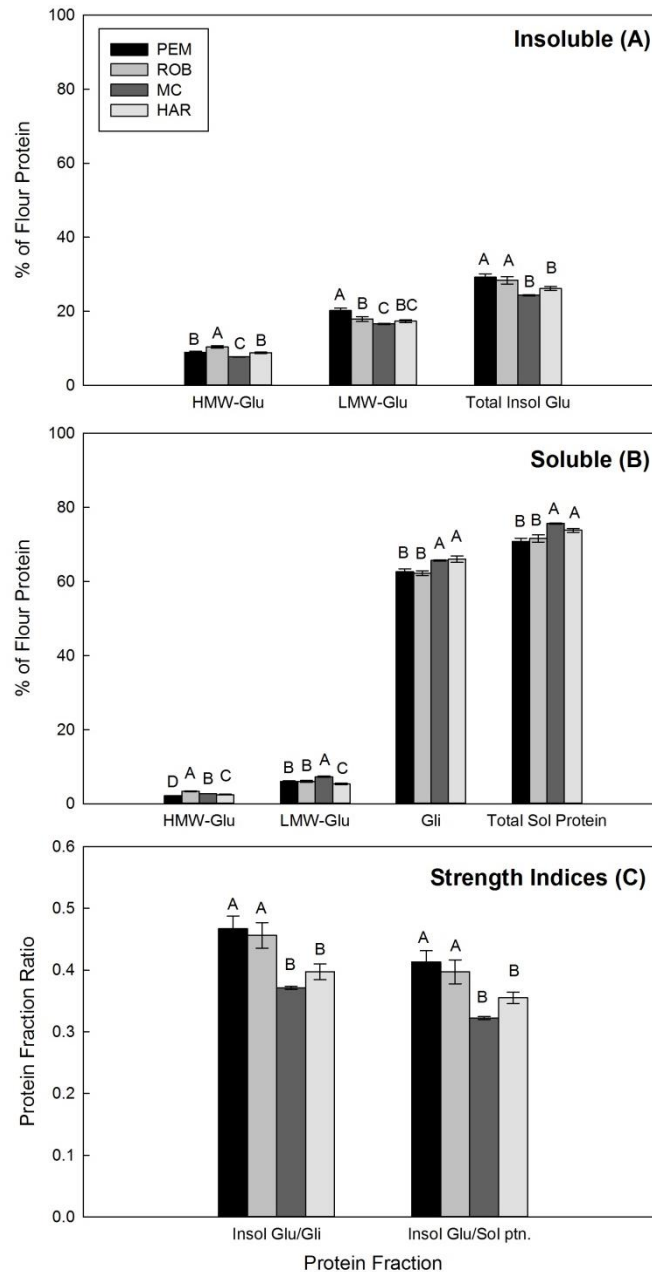


Figure 3.2 Gluten protein fractions of CWRs cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC], and Harvest [HAR]) giving the insoluble gluten fractions (A), soluble gluten fractions (B) and strength indices (C). Data represents mean values  $\pm$  one standard deviation (n=3). Different letters represent significant differences ( $p < 0.05$ ) within each fraction.

given that Pembina proves to be the strongest dough producing flour cultivar in low sodium environments and has the lowest dough stickiness.

The soluble protein fractions given in Figure 3.2B have a weakening effect on the gluten network as it is dominated by gliadins, which are known to impart plasticity in the dough, as well as the soluble glutenins (HMW and LMW), which are of lower molecular weight than the HMW and LMW glutenins found in the insoluble fraction. It can be seen that the weaker flour cultivars of McKenzie and Harvest contain a higher amount of total soluble proteins as well as gliadins (~75% & ~66% respectively), whereas the stronger cultivars of Pembina and Roblin have a lower amount of total soluble proteins as well as gliadins (~ 71% & ~62% respectively).

In Figure 3.2C it can be seen that Pembina and Roblin contain a higher ratio of insoluble glutenin/gliadin and insoluble glutenin/soluble protein than McKenzie and Harvest which results in these flours being weaker than Pembina and Roblin. The ratio of the insoluble glutenin to total soluble protein includes the weakening effect of all the soluble proteins (gliadins, soluble HMW & LMW glutenin subunits, albumins and globulins). In general, with a greater amount of insoluble glutenin subunits the stronger the dough, inversely the greater amount of soluble protein the weaker the dough. The ratio of insoluble glutenin/gliadin displays the ratio of the strengthening insoluble glutenin subunits vs the monomeric gliadins which impart a weakening effect; it is a more specific look at the effect of the protein fractions. These ratios describe well the gluten index for all flour cultivars (Table 3.1), the solvent retention capacity data (associated with lactic acid) (Table 3.3) and gluten performance index (Table 3.3) where again Pembina and Roblin have higher gluten indices, protein solvent retention and gluten performance indices than McKenzie and Harvest.

### **3.4.2 Rheological properties**

The empirical rheological characteristics for each CWRS flour were examined and given in Table 3.4. It should be noted, that although both farinographs and mixographs were used to measure dough development, it is virtually impossible to directly compare the findings due to differences in sample geometry and the inability to replicate the same stress-strain relationship between instruments (Dobraszczyk & Morgenstern, 2003). However, the two techniques are still widely considered to provide useful information concerning the mixing and baking quality of

Table 3.4 Empirical rheological characteristics of flours from CWRS wheat cultivars Pembina, Roblin, McKenzie and Harvest. Data represents the mean values  $\pm$  one standard deviation (n = 2).

Empirical Rheology <sup>1</sup>	Flours			
	Pembina	Roblin	McKenzie	Harvest
<b>Farinograph</b>				
FAB (% to 14% w.b.)	61.5 $\pm$ 0.3 <sup>a</sup>	65.3 $\pm$ 0.1 <sup>c</sup>	63.3 $\pm$ 0.7 <sup>b</sup>	64.9 $\pm$ 0.1 <sup>bc</sup>
DDT (min)	6.3 $\pm$ 0.4 <sup>b</sup>	7.7 $\pm$ 0.2 <sup>c</sup>	4.6 $\pm$ 0.1 <sup>a</sup>	5.2 $\pm$ 0.3 <sup>ab</sup>
MTI (BU)	20.5 $\pm$ 2.1 <sup>a</sup>	14.5 $\pm$ 2.1 <sup>a</sup>	31.0 $\pm$ 9.9 <sup>a</sup>	33.0 $\pm$ 5.7 <sup>a</sup>
STA (min)	8.9 $\pm$ 0.9 <sup>a</sup>	16.9 $\pm$ 1.3 <sup>b</sup>	6.1 $\pm$ 0.9 <sup>a</sup>	5.3 $\pm$ 0.9 <sup>a</sup>
<b>Mixograph</b>				
BA (%)	62.5	64.3	63.4	63.4
MDT (min)	3.23 $\pm$ 0.02 <sup>c</sup>	2.73 $\pm$ 0.05 <sup>a</sup>	3.01 $\pm$ 0.00 <sup>b</sup>	2.68 $\pm$ 0.06 <sup>a</sup>
PDR (%)	51.12 $\pm$ 2.02 <sup>ab</sup>	56.68 $\pm$ 4.02 <sup>b</sup>	45.34 $\pm$ 0.26 <sup>a</sup>	47.41 $\pm$ 0.20 <sup>a</sup>
BWPR (%)	27.65 $\pm$ 1.41 <sup>a</sup>	33.87 $\pm$ 10.38 <sup>a</sup>	21.31 $\pm$ 0.76 <sup>a</sup>	19.99 $\pm$ 0.79 <sup>a</sup>
RBD (%)	1.50 $\pm$ 0.82 <sup>a</sup>	1.83 $\pm$ 0.67 <sup>a</sup>	1.96 $\pm$ 0.49 <sup>a</sup>	1.97 $\pm$ 0.43 <sup>a</sup>
BWBD (%)	7.89 $\pm$ 4.07 <sup>a</sup>	11.01 $\pm$ 6.58 <sup>a</sup>	3.71 $\pm$ 0.21 <sup>a</sup>	3.25 $\pm$ 0.00 <sup>a</sup>
WIP (%tq. min)	117.90 $\pm$ 6.68 <sup>c</sup>	103.67 $\pm$ 2.40 <sup>bc</sup>	97.40 $\pm$ 2.72 <sup>ab</sup>	85.37 $\pm$ 5.62 <sup>a</sup>

<sup>1</sup>Abbreviations: Farinograph water absorption (FAB); dough development time (DDT); mixing tolerance index (MTI); stability time (STA); Barbender units (BU); baking absorption (BA); mixograph development time (MDT); peak dough resistance (PDR); bandwidth at peak dough resistance (BWPR); bandwidth breakdown 1 min after peak (BWBD); resistance breakdown 1 min after peak (RBD); work input to PDR (WIP); and torque (tq).

<sup>2</sup>Different letters represent significantly different values (p<0.05) within a row.

flours and can be used as a tool to screen flours for their dough forming qualities (Dobraszczyk & Morgenstern, 2003).

#### *3.4.2.1 Rheological properties using a farinograph*

Overall, the effect of flour cultivar on farinograph water absorption was found to be significant ( $p < 0.01$ ). Pembina showed the lowest FAB (~61%) of the four flour cultivars ( $p < 0.05$ ) most likely due to its lower protein content relative to the other flours. In general, flours with higher amounts of gluten proteins tend to have higher FAB values. In the present study, McKenzie/Harvest (FAB = ~64%) were found to have higher values than Pembina, but significantly lower values than Roblin (65%) ( $p < 0.05$ ) (Table 3.4); following a similar pattern as the total protein levels found within the flours (Table 3.1). The dough development time (DDT) gives an indication of optimum mixing time to reach maximum consistency of 500 BU (Wheat Marketing Centre, 2008). Findings indicate that Roblin had this highest DDT (~7.7 min), which was significantly higher than Pembina/Harvest (DDT = ~5.7 min), and that of McKenzie (4.6 min) ( $p < 0.05$ ) (Table 3.4). Roblin exhibiting the longest DDT is most likely explained by Roblin having the highest protein content and high quality gluten, as indicated by the gluten index and gluten performance index. Pembina having a shorter mixing time than Roblin even though both are strong cultivars could be due to its lower protein content, however it exhibits high gluten quality. The mixing tolerance index (MTI) gives an indication of degree of dough softening during mixing (Wheat Marketing Centre, 2008); dough with lower MTI values indicate stronger doughs. However, MTI in the current experiments showed high variability among the samples, leading to no significant difference between flours ( $p > 0.05$ ) (Table 3.4). Furthermore, the stability time (STA) gives an indication of dough strength by measuring the time the dough maintains its maximum consistency of 500 BU (Wheat Marketing Centre, 2008). Roblin was found to show the greatest STA (~17 min), which was significantly higher than the other three flours which had a STA similar in magnitude (~7 min) ( $p < 0.05$ ) (Table 3.4). Based on the farinograph results, dough prepared from the stronger flour (e.g., Roblin) would require a greater amount of work energy (i.e., longer mixing and development times) than a weaker flour (e.g., Harvest) and would be less sensitive to over-mixing.

#### 3.4.2.2 Rheological properties using a mixograph

The rheological properties of dough prepared from the four different flours using a mixograph are given in Table 3.4. Baking absorption was calculated based on the formula for optimum absorption (AACC International, 1999). BA values for all flours ranged between 62.5 and 64.3%. The mixograph development time (MDT) gives an indication of the optimum mixing time which is the time it takes for the dough to reach maximum consistency (Wheat Marketing Center, 2008). Weak gluten flour has shorter peak times than strong gluten flour (Wheat Marketing Centre, 2008). MDT values were found to be significantly influenced by flour cultivar ( $p < 0.001$ ); where MDT values increased from ~2.7 min for Harvest/Roblin, to 3.01 min for McKenzie, and then again to 3.23 min for Pembina (Table 3.4). The peak dough resistance (PDR) refers to the percentage of torque at peak development, greater torque is usually indicative of a stronger flour. Overall, PDR values were found not to be influenced by flour cultivar (~50%) (Table 3.4). The values for PDR in the present study were higher than those found by Zghal et al. (2001) for two CWRS flours which were closer to ~35% and 39%, which may be reflective of the fact that Zghal et al. used a 2 g mixograph in their study versus a 10 g in the present study. The bandwidth at the peak dough resistance (BWPR) refers to percent of the full scale torque at peak dough resistance and wider more wild looking bandwidths show the torque spikes, the greater amount of torque necessary to mix the dough the stronger the dough (Hazelton & Walker, 1997). Bandwidth breakdown 1 min after peak (BWBD) gives an indication of dough weakening or the doughs stability (rate of dough breakdown) with respect to the change in the bandwidth from PDR (Hazelton & Walker, 1997; Walker & Walker, 2004). The resistance breakdown 1 min after the peak (RBD) is also an indication of dough weakening or stability with respect to the change of the curve's height (amount of torque) from PDR (Hazelton & Walker, 1997; Walker & Walker, 2004). For the BWPR, BWBD and RBD values, no significant effect of flour cultivar was observed ( $p > 0.05$ ) (Table 3.2). In the case of the work input (WIP), it was observed that WIP for Pembina was higher than Harvest, ~118% vs. ~85% respectively ( $p < 0.05$ ), whereas Roblin (~104%) and McKenzie (~97%) were more intermediate, indicating that Pembina requires a greater amount of energy to bring the dough to its optimum dough development (Table 3.4).

#### 3.4.2.3 Dough extensibility

Extensigraphs are used for classifying flour strength, as weak, medium, strong or very strong dough based on the dough's resistance to extension and extensibility, both of which can influence the final loaf volume of the bread (Delcour & Hoseney, 2013b). Resistance to extension provides a measurement of the dough strength with a higher resistance to extension indicating a greater amount of force needed to stretch the dough, which is typically associated with the glutenin proteins (Delcour & Hoseney, 2013b). In contrast, the dough's extensibility gives an indication of the dough's ability to stretch without breaking, which is typically associated with the gliadin proteins and their ability to induce plasticity to the dough (Delcour & Hoseney, 2013b). In Figure 3.3A it can be seen that the effect of flour cultivar on the resistance to extension is highly significant ( $p < 0.001$ ). The overall trend is that Pembina has the highest resistance to extension (0.31 N) followed by Roblin/McKenzie (~0.24 N) and then Harvest (0.18 N). This trend is similar to that found for work input to peak development (Table 3.4). In Figure 3.3B the effect of flour cultivar on the extensibility was also found to be weakly significant ( $p < 0.05$ ). For extensibility data, only Roblin and Harvest were found to be significantly different from each other ( $p < 0.05$ ), with dough prepared from Roblin (49.7 mm) showing greater extensibility than those prepared with Harvest (44.1 mm).

The ratio of the resistance to extension (R) to extensibility (E) of the dough can be a good indicator of final loaf volume. Higher ratios tend to lead to very elastic dough (i.e., strong gluten network), whereas lower ratios typically lead to more viscous doughs (i.e., weak gluten network) (Delcour & Hoseney, 2013b). In Figure 3.4, the effect of flour cultivar on the R/E ratio was found to be highly significant ( $p < 0.001$ ). Pembina showed the highest ratio of R/E of the four flours (0.0069 N/mm), followed by Roblin (0.0051 N/mm), and then McKenzie (0.0049 N/mm) and Harvest (0.0041 N/mm). However, Roblin, McKenzie and Harvest were not statistically different ( $p > 0.05$ ). Findings suggest that Pembina has strong gluten networks, whereas Roblin, McKenzie and Harvest form comparably weaker ones. However, Roblin is most likely the stronger of the three.

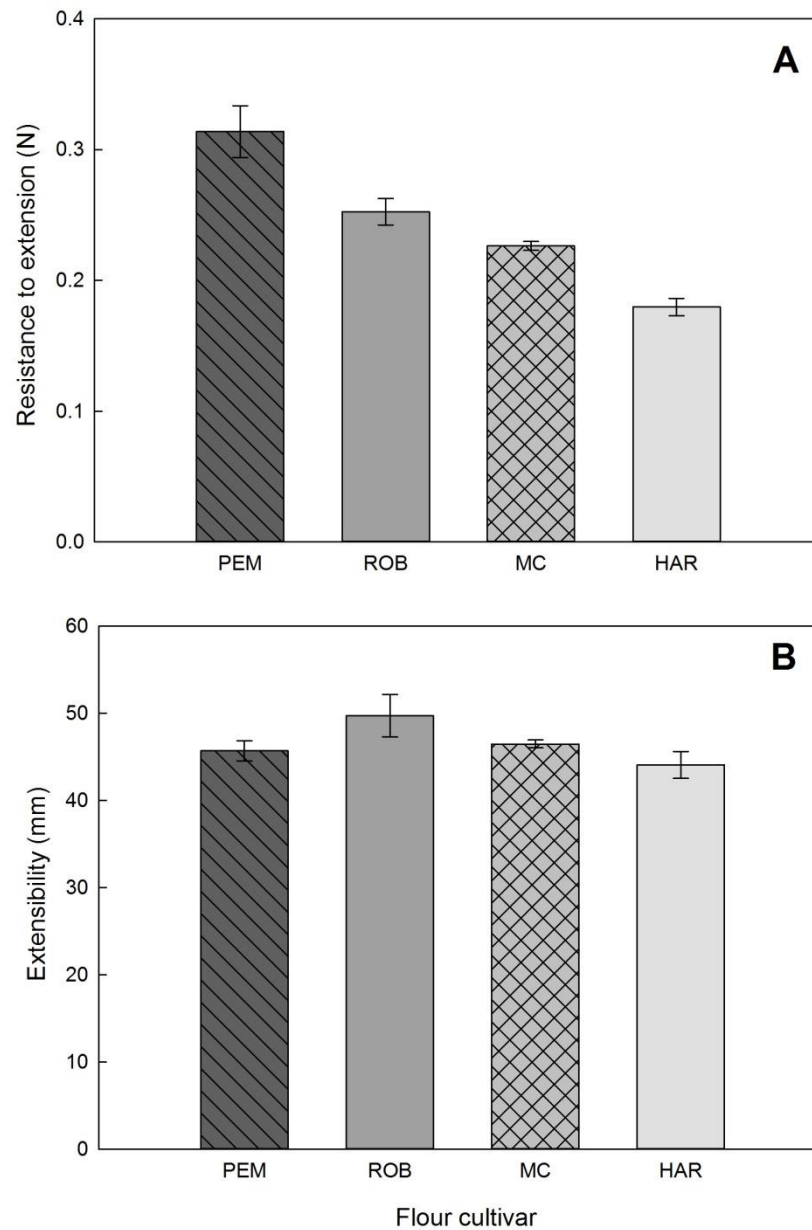


Figure 3.3 Resistance to extension (N) and extensibility (mm) values for dough prepared using different CWRS wheat cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC] and Harvest [HAR]) with 2% NaCl. Data represents the mean  $\pm$  one standard deviation (n = 3).



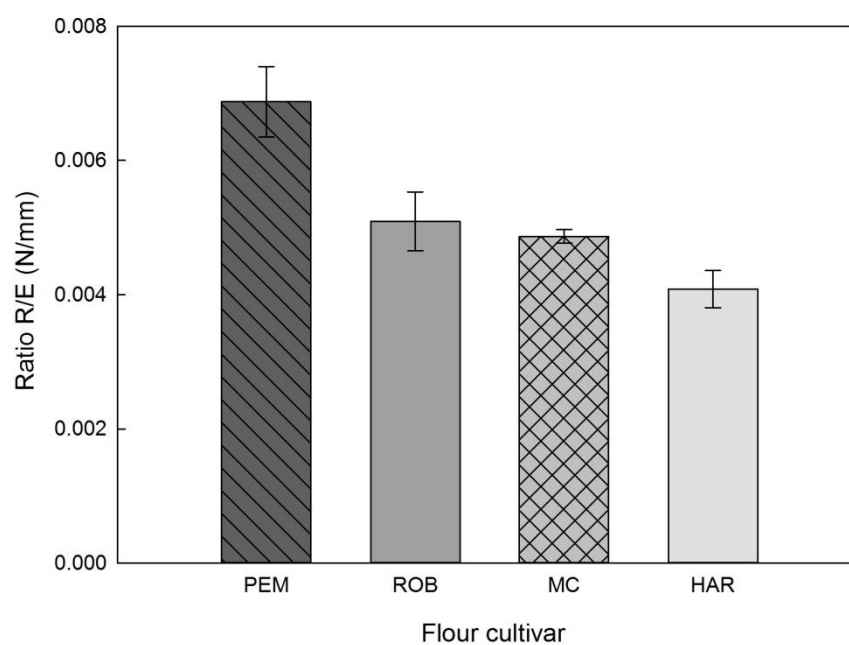
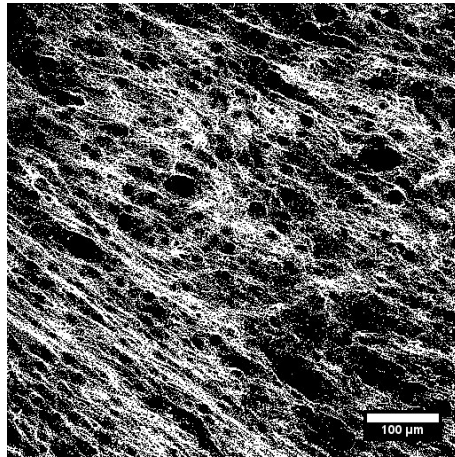


Figure 3.4 Ratio of resistance to extension (N) [R] and extensibility (mm) [E] values for dough prepared using different CWRS wheat cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC] and Harvest [HAR]) with 2% NaCl. Data represents the mean  $\pm$  one standard deviation (n = 3).

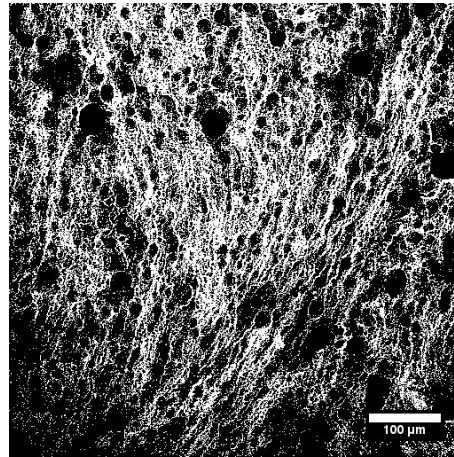
### 3.4.3 Confocal laser scanning microscopy (CLSM)

Confocal laser scanning micrographs were taken to better understand how the structure of the gluten network relates to dough strength. From the micrographs (Figure 3.5), it appears that doughs prepared with Pembina, Roblin and McKenzie flours all formed elongated gluten polymeric strands to create a more continuous network with directional organization. In contrast, doughs prepared using Harvest flours formed shorter gluten strands with a porous structure with random orientation. CLSM images were assessed in order to quantify differences in dough morphology among the different cultivars, and are reported in Table 3.5. Particle count in the present study was equated to the size of the gluten aggregates, where larger aggregates would have a corresponding lower particle count. In the present study, dough prepared using Pembina was found to have a significantly higher particle count (5051) and lower average particle size ( $\sim 31 \mu\text{m}^2$ ) than those prepared with Harvest flour (3783,  $\sim 44 \mu\text{m}^2$ ) indicating that there are fewer large gluten aggregates and more individual defined gluten polymers with directional orientation (Table 3.5). Doughs formed with Harvest are presumed to have larger, more randomly oriented aggregates, and hence a more porous structure. Particle count and average particle sizes in doughs prepared from Roblin and McKenzie were similar, and in-between that of Pembina and Harvest.

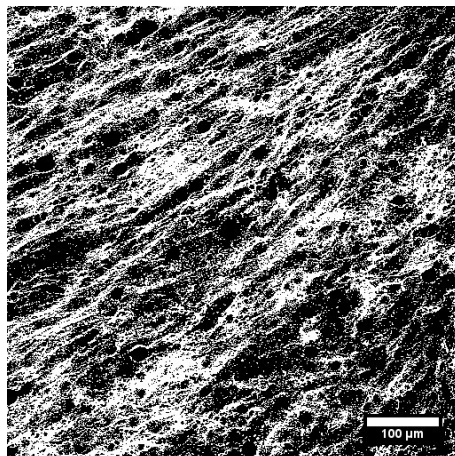
The total particle area, circularity and fractal dimension did not provide good indicators of structural differences within the dough. Jekle and Becker (2011) reported that the parameters of particle count and circularity showed low significance; however they found that total particle area, average size and fractal dimension showed significant differences within their samples. Differences in reliability of image analysis parameters could be due to the difference in dough samples. Samples presented here were examined utilizing different wheat cultivars however Jekle and Becker were testing the effect of different water additions, which, based on their results appears to have a greater effect on the image analysis results of the microstructure and thus greater detectability. Peighambardoust and others (2006) determined that the image analysis utilized for their dough samples was not sufficient to explain the differences in dough development despite reported correlations by two other studies. Jekle and Becker reported that a decrease in total particle area in samples with greater water addition was associated with a less developed gluten network due to the hindrance of the expanded and congregated starch granules



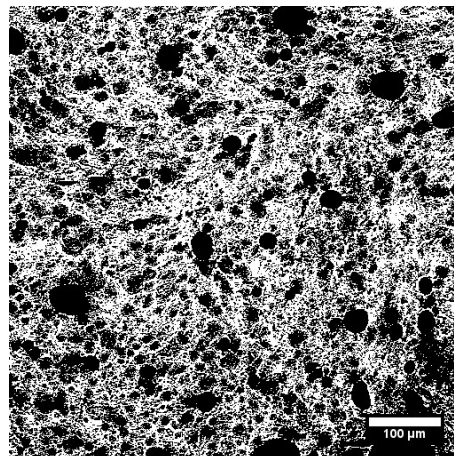
A) PEM



B) ROB



C) MC



D) HAR

Figure 3.5 Confocal laser scanning microscopy images for dough prepared using different CWRS wheat cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC] and Harvest [HAR]) with 2% NaCl.

Table 3.5 Image analysis of confocal laser scanning microscopy of doughs prepared using CWRS wheat cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC], Harvest [HAR]) with 2% NaCl. Values represent mean of 6 images  $\pm$  one standard deviations.

Flour Cultivar	<sup>1</sup> Particle Count	<sup>2</sup> Total Particle Area (10 <sup>4</sup> $\mu\text{m}^2$ )	<sup>3</sup> Average Particle Size ( $\mu\text{m}^2$ )	<sup>4</sup> Circularity	<sup>5</sup> Fractal Dimension
PEM	5071 $\pm$ 548 <sup>b</sup>	15.57 $\pm$ 1.68 <sup>b</sup>	31.19 $\pm$ 5.94 <sup>ab</sup>	0.90 $\pm$ 0.02 <sup>a</sup>	1.90 $\pm$ 0.01 <sup>a</sup>
ROB	4387 $\pm$ 645 <sup>ab</sup>	15.92 $\pm$ 1.95 <sup>b</sup>	37.29 $\pm$ 8.78 <sup>bc</sup>	0.89 $\pm$ 0.01 <sup>a</sup>	1.90 $\pm$ 0.02 <sup>a</sup>
MC	4828 $\pm$ 384 <sup>b</sup>	11.88 $\pm$ 2.27 <sup>a</sup>	38.95 $\pm$ 3.06 <sup>a</sup>	0.89 $\pm$ 0.01 <sup>a</sup>	1.91 $\pm$ 0.10 <sup>a</sup>
HAR	3783 $\pm$ 402 <sup>a</sup>	16.64 $\pm$ 0.52 <sup>b</sup>	44.50 $\pm$ 5.74 <sup>c</sup>	0.88 $\pm$ 0.01 <sup>a</sup>	1.92 $\pm$ 0.01 <sup>a</sup>

NOTES: <sup>1</sup>Particle count is associated with degree of protein aggregation. <sup>2</sup>Total particle area is associated with total area covered by the particles counted. <sup>3</sup>Average particle size is associated with the average size of all the particles counted. <sup>4</sup>Circularity is associated with the shape of the particles counted (value closer to 1 = more circular and value closer to 0 = more elongated). <sup>5</sup>Fractal dimension is associated with the degree of pattern or complexity. Parameters were defined based on the Image J analysis guide. Different letters within a column represents significantly different values  $p < 0.05$ .

due to the excess water added. From their findings it would have been expected that in the present study Pembina would have a greater total area than Harvest given that Pembina appears to form a more continuous gluten network than Harvest and thus a more developed gluten network. However this was not the case, Pembina, Roblin and Harvest all had similar total areas. The reason for this could be that although Pembina had a more developed network than Harvest, both the more structured gluten polymers within Pembina and the aggregated gluten polymers within Harvest cover the same area in a different manner. Therefore this would explain the lack of differences for the fractal dimension measurements and the fact that Harvest has the greatest average particle size. The findings within the CLSM micrographs follow the results for the resistance to extension in Figure 3.3A where the effect of flour cultivar was highly significant

with Pembina showing the highest resistance to extension followed by Roblin/McKenzie and then Harvest having the lowest indicating the weakest gluten network formation.

### **3.5 CONCLUSIONS**

Although the majority of the compositional data was similar among the different CWRS cultivars examined, the most notable differences were with the gluten quantity/quality (subunit composition) and damaged starch content. Pembina flour was found to have the lowest protein content of the four flour cultivars but had the greatest resistance to extension and the most elongated gluten protein polymers with unidirectional organization. These findings suggest that gluten content plays less of a role in gluten strength relative to gluten quality. Examination of gluten quality using the gluten index and analysis of the gluten composition with respects to the insoluble glutenins vs the gliadins revealed that the two stronger dough producing flours of Pembina and Roblin had higher gluten indices than those of McKenzie and Harvest. Pembina was found to have a higher amount of low molecular weight glutenin (insoluble) proteins relative to Roblin, which had higher amounts of high molecular weight glutenin (insoluble) proteins, possibly accounting for the differences in dough strength in the present study. McKenzie and Harvest flours were higher in gliadin contents than Pembina and Roblin. Furthermore, the level of damaged starch was presumed to have played a role in the gluten network formation and strength given that it competes with the gluten proteins for hydration. With extensive damaged starch there can be a detrimental impact on the gluten network formation as is seen with McKenzie and Harvest which had higher damaged starch quantities and gluten that is of lower quality than that of Pembina and Roblin and thus are presumed to be unable to overcome the weakening impact of the higher level of damaged starch.

### **3.6 LINKAGE TO CHAPTER 4**

Findings from this study will help provide foundational knowledge and determine the effect of differences in flour composition and gluten protein composition/quality on dough stickiness and handling within low sodium formulations. The differences in the insoluble HMW and LMW glutenin subunits and amount of gliadins within the flour may provide the explanation as to what is occurring in the gluten network formation with the decrease in sodium chloride and

why there are differences in stickiness between the flour cultivars with reduced sodium formulations.

#### **4. EFFECT OF NaCl LEVEL ON THE HANDLING PROPERTIES OF DOUGH PREPARED FROM DIFFERENT CANADA WESTERN RED SPRING WHEAT CULTIVARS**

##### **4.1 ABSTRACT**

The dough handling properties for four CWRS wheat cultivars (Pembina, Roblin, McKenzie and Harvest) were investigated as a function of NaCl (0-4%) level. Specifically, the dough rheology (oscillatory and creep recovery), extensibility, stickiness and water mobility were studied. In terms of dough rheology, the loss tangent was found to be similar for doughs prepared with Roblin, McKenzie and Harvest, and weaker than that of Pembina. In all cases, dough became stronger in a curvilinear fashion as the NaCl levels increased from 0 to 4%. Further, the amount of deformation in the dough decreased with increasing NaCl levels indicating that the gluten network became stronger as it was able to resist the imposed stress. For extensibility, increasing the levels of NaCl resulted in an increase in resistance to extension for all flour cultivars. For stickiness at the 0 and 2% NaCl, doughs prepared with Pembina and Roblin showed the least stickiness relative to McKenzie and Harvest. Water association as a function of NaCl level was determined within the doughs of the four different cultivars as being either free water or associated with the starch-fraction or gluten-fraction. Findings indicated that with the addition of NaCl there was a decrease of free water among the different cultivars and an increase in the water associated with the starch-fraction. Dough morphology followed along with the trends of rheology with the stronger dough producing cultivars creating more elongated protein polymers with a unidirectional network whereas the weaker cultivars created porous multidirectional networks. Overall, Pembina and Roblin formed stronger gluten networks than McKenzie and Harvest, where the sensitivity of NaCl was found to be cultivar dependent. Stickiness was more notable in the weaker cultivars than the stronger.

## 4.2 INTRODUCTION

Sodium chloride is a common additive to processed foods for flavour, functionality, and preservation. Its reduction in food products is now being either mandated or voluntarily recommended by governments due to the adverse health effects of high sodium diets. However, in some foods sodium reduction is less about the negative effect of loss of flavour and more about the loss of functionality. Bread (and other cereal products) account for ~30% of sodium intake and is commonly consumed daily (Farahnaky & Hill, 2007; Miller & Hosene, 2008; Lynch et al., 2009; Belz et al., 2012). Sodium chloride plays a number of roles aside from providing flavour to a loaf of bread; it is necessary for enhancing the functional properties of strength and handling of the dough (Miller & Hosene, 2008; Uthayakumaran, et al., 2011). Salt achieves this through its impact on the gluten protein network formation during mixing; it increases dough development through increased mixing time allowing for increased protein-protein interaction and therefore increased strength; it controls the rate of yeast fermentation, therefore affecting the rate of gas production (Uthayakumaran et al., 2011; Belz et al., 2012). Through its impact on dough handling properties salt leads to an improvement in loaf volume and crumb grain quality. With the reduction of sodium chloride there are detrimental effects on bread loaf quality. More specifically, sodium reduction negatively impacts dough rheology and handling properties in the mixing stage due to a sticky dough phenomenon causing major processing issues as well as a poor quality final product (Farahnaky & Hill, 2007).

Dough stickiness occurs when the adhesive forces (interactions between the dough surface and the mixing surface) are high and the cohesive forces (interactions within the dough i.e., protein-protein interactions) are low (Adhikari et al., 2001; van Velzen et al., 2003). Previous studies have determined the following factors are linked to the phenomenon of dough stickiness: flour extraction, amount of water soluble pentosans, protein composition,  $\alpha$ -amylase activity, and proteolytic activity (Chen & Hosene, 1995a; Hosene & Smewing, 1999; van Velzen et al., 2003). Along with these factors, processing (i.e., mixing time/speed, energy input, temperature, relaxation time) and formulation (i.e., level of hydration, ingredients, quality of flour) factors can contribute to dough stickiness (van Velzen et al., 2003, Belz et al., 2012). Bread dough is comprised of three phases; the first being the viscoelastic gluten network, the second being the embedded starch granules, free water and other water soluble flour components; and the third being the entrapped air and CO<sub>2</sub> (Jekle & Becker, 2011). All of these



phases have the ability to affect the microstructure formation and therefore ultimately impact the dough handling properties and dough stickiness.

The viscoelastic gluten network formation is of the utmost importance in producing strong dough with good handling properties and determining final bread quality. Without the addition of salt the gluten proteins carry an overall net positive charge as they are below their isoelectric point (pH 7.5) within the flour-water system (pH ~6.0) (Gennadios et al., 1993; Miller & Hoseney, 2008). Due to the net positive charge the proteins repel one another and become more hydrated in the flour water-system causing decreased mixing times due to decreased protein-protein interactions which results in a weaker and stickier dough (Miller & Hoseney, 2008). However when salt is added to the flour-water system it serves to shield the charged sites on the protein's surface enabling the proteins to interact and aggregate through hydrophobic interactions and hydrogen bonds. Increased protein-protein interactions occur, due to a slower, and therefore decreased protein hydration which results in a dough formation that is stronger and non-sticky (Preston, 1989; Butow et al., 2002; Miller & Hoseney, 2008; Uthayakumaran et al., 2011).

Dobraszczyk (1997) studied the rheological basis of dough stickiness to find that the storage modulus was negatively correlated with stickiness and sticky doughs had faster relaxation times over non-sticky doughs. This led to the conclusion that stickiness is a process controlled by rheology. Correlations between sensory stickiness and rheological properties of compression, relaxation and tension of doughs were also reported by Wang and others (1996). Measuring the rheological properties of the dough gives an indication of the cohesive forces within the dough and thus if cohesive forces are low it results in sticky dough (Hoseney & Smewing, 1999). Hoseney and Smewing also stated that factors which affect the surface tension of water also have an effect on stickiness. In previous studies utilizing similar small deformation rheological measurements there have been conflicting results on the effect of NaCl on the storage modulus ( $G'$ ) (Lynch et al., 2009; Beck et al., 2012a; McCann & Day, 2013; Tuhumury et al., 2014). Some found that the  $G'$  increased with decreasing NaCl (Salvador et al., 2006; Lynch et al., 2009; McCann & Day, 2013; Tuhumury et al., 2014) whereas others found the  $G'$  to decrease with decreasing NaCl (Larsson, 2002; Beck et al., 2012a). Within these studies it is stated that differences in the findings may stem from differences in protein content and quality. Different wheat cultivars, with different protein quantity/quality, interact differently with regard

to sensitivity to NaCl (Butow et al., 2002; Lynch et al., 2009; Tuhumury et al., 2014). These differing effects of NaCl on different protein qualities would also have differing effects on protein hydration and therefore other flour component hydration, all of which could affect dough stickiness.

The overall goal of this research was to examine the relationship between dough rheology and stickiness, and bound vs unbound water within the doughs prepared from four different CWRS wheat cultivars under reduced sodium conditions.

## **4.3 MATERIALS AND METHODS**

### **4.3.1 Materials**

Four CWRS wheat flour cultivars (Pembina, Roblin, McKenzie, and Harvest) were kindly provided the Crop Development Centre at the University of Saskatchewan (Saskatoon, SK). The four cultivars were grown in plots at the University of Saskatchewan's Kemen Crop Research Farm under rain-fed conditions on fallowed land. The previous crops in the rotation were canola, pea, and barley. All cultivars were seeded May 21, 2013 and each cultivar was grown in a 2.4 m wide by 15.2 m long plot with row spacing of 17.8 cm. The herbicide, fungicide and fertilizer used were: BuctrilM (1.0 L/ha) [applied June 11, 2013], Headline (0.4 L/ha) [applied June 28, 2013], and 56.0 kg/ha 23-23-0-10 [applied with seed], respectively. For seed conditioning: 13/64 round holed screen to scalp, 5.5 X ¾ slotted and 12 triangle buckwheat screens to sift were used. The cleaned amounts for each cultivar were: Pembina-40 kg, Roblin-48 kg, McKenzie-42 kg and Harvest-60 kg. Pembina was developed at the Canada Department of Agriculture Research Station (Winnipeg, MB, Canada) by the Rust Area Project Group; it is unlikely to be grown outside of the rust area and is best suited to the Red River Valley (Campbell, 1963). Pembina is a cultivar that has an excellent baking quality, a yield comparable to that of Selkirk grown in the Red River Valley, slightly more rust resistance than Selkirk and has a one day earlier maturation than Selkirk (Campbell, 1963). Roblin is a cultivar for the eastern prairies of Canada that was developed at the Agriculture Canada Research Station (Winnipeg, MB, Canada) it has a similar quality to Marquis, and is a high-protein, early-maturing and rust-resistant cultivar (Campbell & Czarnecki, 1987). McKenzie was developed by the Saskatchewan Wheat Pool (Agricultural Research and Development, Saskatoon, SK, Canada) and has a high grain yield; early maturity; high test weight; high falling number;

resistance to stem rust, leaf rust, and common bunt; and has intermediate resistance to Fusarium head blight (Graf et al., 2003). McKenzie is a cultivar suited to all areas of the Canadian Prairies (Graf et al., 2003). Harvest is a cultivar that was developed by Agriculture and Agri-Food Canada (Cereal Research Centre, Winnipeg, MB, Canada) that is suitable to the wheat-producing regions of the Prairies (Fox et al., 2010). Harvest has the qualities of high-yield, high test weight and preharvest sprouting resistance (Fox et al., 2010). Milling of the four selected wheat cultivars was done on a Buhler MLU202 Experimental Mill according to the American Association of Cereal Chemists International approved method 26-21.02. Prior to milling the grain was tempered to a moisture of 15.5% for ~ 16 hrs.

Deionized and distilled water (DDH<sub>2</sub>O) was used in all dough formulations. Rhodamine B was purchased from Sigma Aldrich (Oakville, ON, Canada). Sodium chloride (NaCl) and paraffin oil were purchased from VWR (Mississauga, ON, Canada) and were ACS reagent grade. The water used in this research was produced from a Millipore Milli-Q<sup>TM</sup> water purification system (Millipore Corp., Milford, MA, USA).

#### **4.3.2 Rheological analysis**

##### *4.3.2.1 Dough preparation*

All dough was prepared using a 10 g mixograph (TMCO National Mfg., Lincoln, NE) utilizing a dough formulation comprised of flour (weight on a 14% moisture basis), water (weight based on farinograph absorption results) and NaCl salt (0-4% on flour weight basis). All doughs were mixed until just after reaching peak time. All doughs were prepared in triplicate.

##### *4.3.2.2 Dough rheology*

Dough rheology was performed according to Jekle & Becker (2011) using an AR-1000 rheometer (TA Instruments, New Castle, USA) equipped with a 40 mm parallel plate fixture (2 mm gap). The temperature was kept constant at 30°C using a Peltier plate temperature system. Dough (~5 g) was weighed out and then placed in-between the two plates. The excess dough that resulted once the parallel plate fixture was lowered was trimmed away using a plastic spatula and paraffin oil was applied to the free surface of the dough to prevent the dough from drying out. A resting period of 10 min was utilized for the dough prior to any measurements being taken. The first test that was run was the oscillatory frequency sweep followed by the creep recovery test.

*Oscillatory frequency sweep:* The frequency was varied from 0.10 to 100.00 Hz at a constant amplitude strain of 0.03% which was determined to be within the linear viscoelastic regime based on preliminary testing. The latter was determined using a strain sweep to determine at which strain the material deviates from linearity. The dynamic storage ( $G'$ ) and loss ( $G''$ ) moduli of the dough samples were measured. Values were reported for 1 Hz. From these measurements the loss factor was calculated using the following equation:

$$\tan \delta = G''(G')^{-1} \quad (\text{Eq. 4.1})$$

*Creep recovery test:* In the creep phase a constant shear stress  $\tau_0$  of 250 Pa at 30°C was applied to the dough sample for the duration of 180 s and then removed ( $\tau_0 = 0$  Pa). The recovery/relaxation phase of the dough was recorded for 360 s. The strain values were collected as a function of time and the final data was given in terms of compliance:

$$J(t) = \gamma(t) \tau_0^{-1} \quad (\text{Eq. 4.2})$$

where  $J$  is the compliance,  $\gamma$  strain, and  $\tau_0$  the constant stress which was utilized during the creep phase. The parameters of the creep phase measurements include the time and stress dependent recoverable shear deformation, the creep compliance  $J_{\max}$  (at  $t=180$  s of the creep phase). The creep recovery compliance  $J_r$  (at  $t=360$  s of the recovery phase) is a measure of the material's elasticity. The recovery compliance describes the mechanical energy that is stored in the sample during the creep phase. The relative elastic part  $J_{el}$  [-] was calculated by utilizing:

$$J_{el} = J_r (J_{\max})^{-1} \quad (\text{Eq. 4.3})$$

#### 4.3.3 Dough Stickiness

Dough stickiness was measured according to the Chen and Hosney method (1995a) using a stable microsystems (SMS) TA.XTplus Texture profile analyzer equipped with a 5 kg load cell, using a Perspex cylinder 25.0 mm probe adhesion fixture and the SMS/Chen-Hosney dough stickiness cell. The dough samples were loaded into the cell and then extruded through the

openings of the mesh screen by turning the dial on the bottom of the cell. A plastic spatula was then utilized to clean the initial extruded dough from the screen surface. To keep the dough height as consistent as possible, the dial was turned two thirds of the way, giving ~ 1 mm of dough height. After extrusion a plastic cover was placed over top of the dough, to reduce moisture loss, as it rested for 30 s. The cover was then removed and the probe was brought down to about 1 mm above the dough surface and then the test was run using a pre-test speed of 0.5 mm/s, test speed of 0.5 mm/s, post speed 10.0 mm/s, distance 15.0 mm, force 40.0 g, time 0.1 s, and trigger force 10.0 g. The force required by the Perspex probe to separate from the dough's surface was recorded as the dough stickiness measurement. Doughs were run in triplicate with each trial containing approximately six runs from one loading of the cell.

#### **4.3.4 Dough extensibility**

Dough extensibility was measured utilizing a stable microsystems (SMS) TA.XTplus Texture profile analyzer equipped with a 5 kg load cell and an SMS/Kieffer rig with modifications to the procedure (Stable Micro Systems, 2005). Settings for the test were as follows: pretest speed of 2.0 mm/s, test speed of 3.3 mm/s, posttest speed of 10.0 mm/s, distance of 100.0 mm and a trigger force of 5.0 g. The dough was not rested before loading into the oiled form. Once the dough was in the dough clamp the dough was rested for 10 min at room temperature (21-23°C). After the resting period the dough strips were then loaded onto the platform and measurements were taken. The measurements taken were dough resistance to extension (N), which is the force at the maximum height of the curve and dough extensibility (mm) which is the length of the curve upon dough breaking.

#### **4.3.5 Differential scanning calorimetry (DSC)**

Freezable water content was determined according to Lu and Seetharaman (2013) utilizing a Q series DSC Q2000 (TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling system and nitrogen as the purge gas. Briefly, dough samples of ~ 10-20 mg were weighed into aluminum DSC pans which were then hermetically sealed before analysis and an empty pan was used as the reference. The reference pan and sample pan were then loaded and equilibrated at 30°C for 5 min. The pans were cooled from 30°C to -40°C at a rate of 10°C/min, held at -40°C for 5 min, followed by heating from -40°C to 40°C at a rate of 10°C/min. The

melting peak enthalpy ( $\Delta H$ ) was obtained using the Universal Analysis 2000 version 4.5A software (TA instruments, New Castle, DE, USA). The freezable water content was calculated from the melting enthalpy peak divided by the enthalpy of pure water (333.55 J/g) and reported on a dry weight basis. Moisture determination was done on each dough in triplicate using a NaCl range of 0-4%, and each flour cultivar (Pembina, Roblin, McKenzie, and Harvest).

#### **4.3.6 Thermogravimetric analysis (TGA)**

Thermogravimetric analysis was performed on dough using TGA Q500 apparatus (TA Instruments, New Castle, DE, USA). Dough samples were prepared using Pembina, Roblin, McKenzie and Harvest flours with NaCl levels ranging from 0-4% on weight/weight basis. Dough samples (~70 mg) were placed on platinum pans and scanned from 25 to 200°C at a rate of 5°C/min. The weight change (which was attributed to moisture loss) and derivative of weight loss as a function of temperature were obtained. The derivative thermogravimetric (DTG) curve was used to differentiate between diffusive water (weakly absorbed free water entrapped near the surface of the dough; more mobile) and more tightly entrapped (more strongly absorbed multilayer water; less mobile) or bound water within the dough as done by Fessas and Schiraldi (2001), Crockett et al. (2011) and Roozendaal et al. (2012). The DTG traces were deconvoluted into Gaussian distributions using PeakFit software (Systat Software, Inc., San Jose, CA, USA) based on the method of Roozendaal et al. (2012) to give an indication of water's association with dough components of starch and gluten.

#### **4.3.7 Confocal laser scanning microscopy (CLSM)**

Confocal laser scanning microscopy was utilized to visualize the gluten network formation of the four different wheat flour cultivars both with (2% NaCl) and without sodium chloride (0% NaCl) and was based off of the method by Jekle and Becker (2011). The doughs were prepared as described above. The fluorescent dye rhodamine B was prepared at a concentration of 0.001% (w/v) in DDH<sub>2</sub>O and was then utilized as the stock water addition to each dough formulation to ensure that the dye was distributed homogeneously throughout the dough during the mixing process. A small sample was then taken from the dough with two plastic spatulas to try to prevent stretching of the dough, the sample was about 4-5 mm in diameter. The dough sample was then loaded on the slide and flattened with the cover slip by

placing the cover slip overtop and then turning the slide over and lightly pushing it down on the table. All samples were loaded this way. The dough was analyzed by a Nikon C2 CLSM microscope (Nikon, Mississauga, ON, Canada) using 20x Plan Fluor (numerical aperture 0.75, Nikon) objective lens. The excitation and emission wavelengths were 543 nm and 625 nm (rhodamine B), respectively. A total of 10 images with 512 x 512  $\mu\text{m}$  pixel resolution were taken for each dough sample from different positions on the  $xy$ -axis. Each dough sample was analyzed in duplicate. Three images of each dough were assessed for particle count, total particle area, average particle size, circularity and fractal dimension using Image J software (National Institutes of Health, Bethesda, MD, USA) based off of Jekle and Becker (2011).

#### **4.3.8 Statistical analysis**

A two-way analysis of variance (ANOVA) was used to show statistical differences for all parameters (e.g.,  $G'$ ,  $G''$ ,  $\tan \delta$ ,  $|G^*|$ ,  $J_{\max}$ ,  $J_{\text{el}}$ , stickiness, resistance to extension, extensibility, freezable water content, max. peak height temp., peak weight loss, total weight loss, relative proportion of peak water loss, peak temp., particle count, total particle area, average particle size, circularity, fractal dimension) as a function of flour cultivar and NaCl level. Since all parameters showed a significant two-way interaction, a subsequent one-way ANOVA using a Scheffe Post-hoc test was performed to test for differences in behaviour with the level of salt within each cultivar. The statistical analysis program IBM SPSS (Armonk, NY, USA) was utilized.

### **4.4 RESULTS AND DISCUSSION**

#### **4.4.1 Dough rheology**

##### *4.4.1.1 Oscillatory shear*

Oscillatory shear rheometry was used to assess the handling properties of dough prepared from the four CWRS wheat cultivars as a function of NaCl levels. Small deformation rheology allows for the examination of the elastic and viscous properties of the dough to be examined in a non-destructive manner, maintaining the internal microstructure of the dough. For all sample treatments the storage modulus (or  $G'$ ) (describing the elastic component of the dough) was greater than the loss modulus (or  $G''$ ) (describing the viscous component of the dough) (Figure 4.1A,B) indicating a highly structured gluten network within the dough. The loss tangent (or  $\tan \delta$ ) represents the ratio of  $G''$  over  $G'$ , where values  $<1$  indicate the material is behaving as an

elastic solid, whereas values  $>1$  indicate the material is behaving more as a liquid. A two-way analysis of variance of  $G'$ ,  $G''$ ,  $\tan \delta$  and complex modulus ( $|G^*|$ ) found that both the effect of NaCl and flour cultivar, along with their interaction made a significant difference ( $p < 0.05$ ). Overall the magnitude of both  $G'$  and  $G''$  was greatest for Pembina followed by McKenzie, Roblin and then Harvest regardless of the NaCl content (Figure 4.1A,B). In the case of  $G'$ , all flour cultivars saw an increase in dough strength as the levels of NaCl were raised from 0 to 4% (Figure 4.1A), although at different rates. Pembina and McKenzie seemed to be more sensitive to changes in NaCl levels than Roblin and Harvest as evident by their steeper slopes. In contrast,  $G''$  was less sensitive to the effect of NaCl level where the magnitudes were found to be constant for all flour cultivars between 0 and 2%, and then increased slightly at 4% NaCl (Figure 4.1B). However, the ratio of the two (i.e.,  $\tan \delta$ ) suggests that the dough properties of Roblin, McKenzie and Harvest were similar, and formed weaker gluten networks than that of Pembina. In all cases, dough became stronger in a curvilinear fashion as the NaCl levels increased from 0 to 4% (Figure 4.1C). The addition of NaCl is believed to shield charges on the amino groups on the gluten proteins, effectively reducing its electric double layer to promote protein-protein aggregation and hydrophobic interactions, and then ultimately a stronger more structured gluten network.

The complex modulus ( $|G^*|$ ) describes the overall resistance to flow of the material in response to imposed oscillatory strain, and is a direct measure of the rigidity or stiffness of the dough. Overall, dough prepared by Pembina displayed the greatest dough rigidity of all the flour cultivars examined, followed by McKenzie and Roblin which were similar, and then Harvest (Figure 4.2). In all cases,  $|G^*|$  was relatively independent of NaCl between 0 and 2%, although both Pembina and McKenzie saw a slight increasing trend over the NaCl level. (Figure 4.2). All dough, regardless of the cultivar, saw an increase in dough rigidity as NaCl increased from 2% to 4% (Figure 4.2).



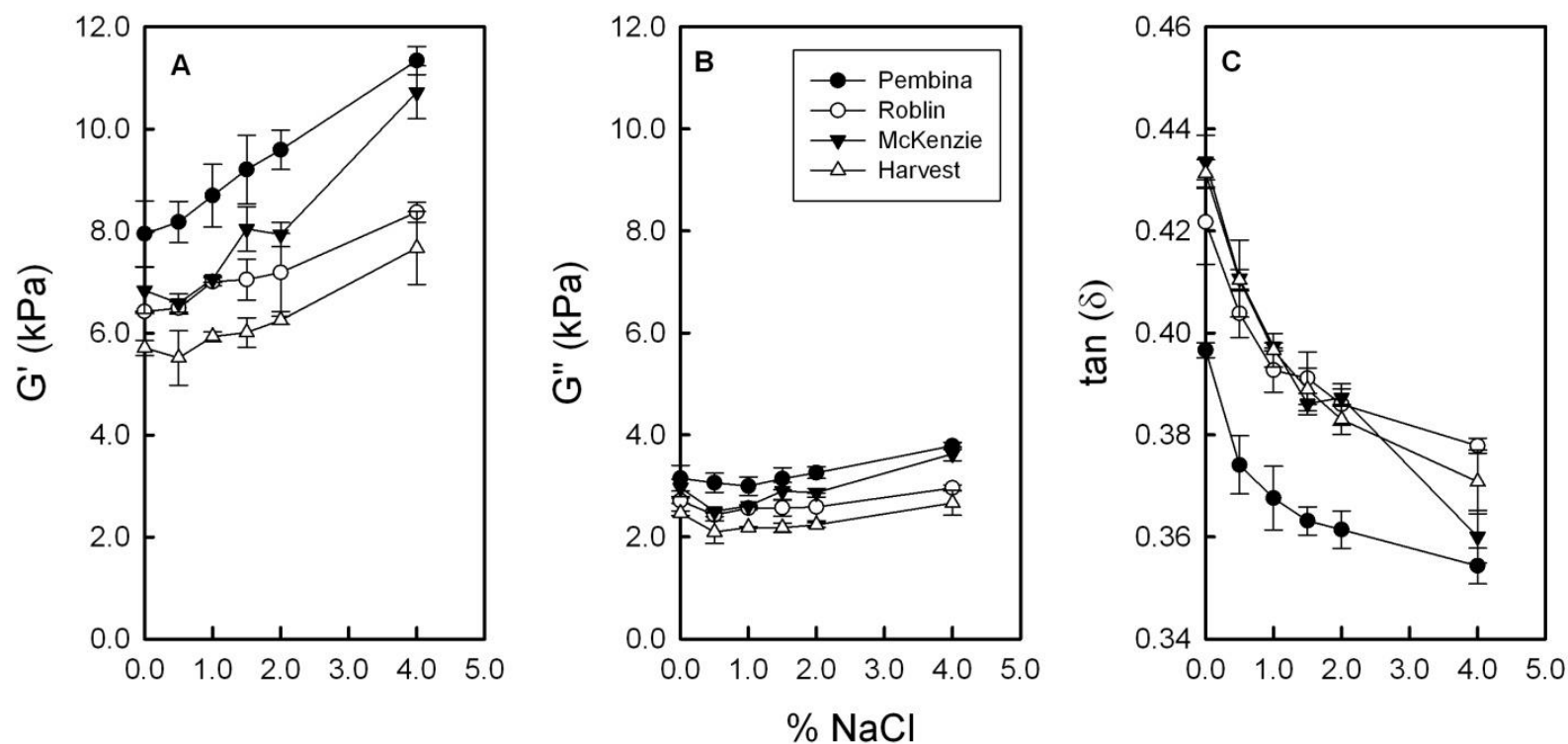


Figure 4.1 Dynamic storage ( $G'$ ) (A) and loss ( $G''$ ) (B) moduli, and  $\tan(\delta)$  (C) at 1 Hz for dough prepared using different CWRS wheat cultivars (Pembina, Roblin, McKenzie and Harvest) as a function of NaCl level. Data represents the mean  $\pm$  one standard deviation (n = 3).

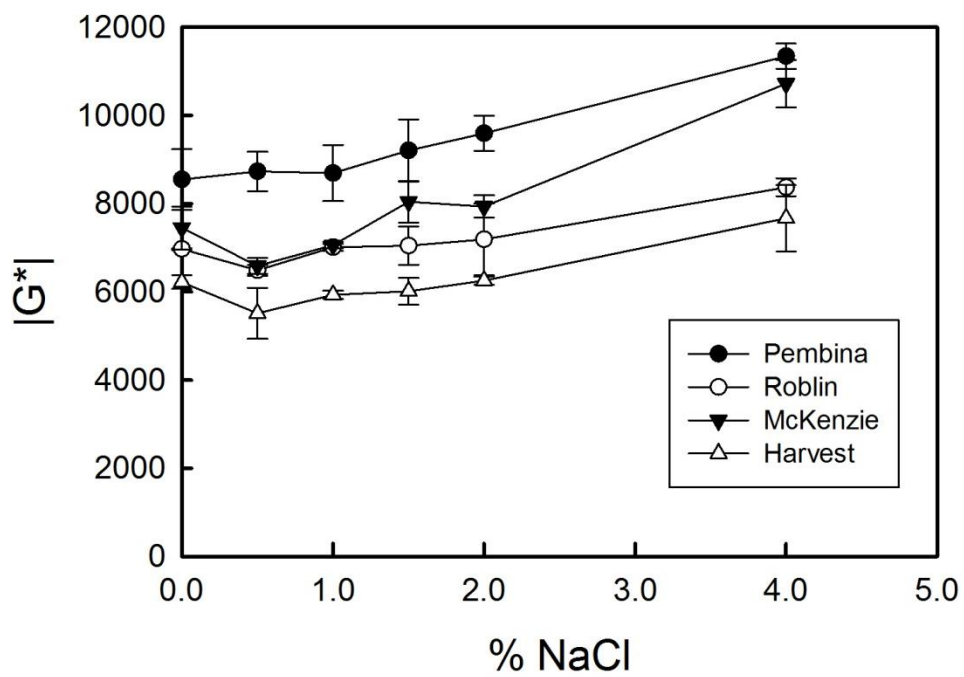


Figure 4.2 Complex modulus ( $|G^*|$ ) at 1 Hz for dough prepared using different CWRS wheat cultivars (Pembina, Roblin, McKenzie and Harvest) as a function of NaCl level. Data represents the mean  $\pm$  one standard deviation ( $n = 3$ ).

#### 4.4.1.2 Creep recovery

Creep recovery measurements were taken to examine the amount of deformation given a constant applied stress that the dough would experience, and then the level of recovery, to give an indication of dough elasticity. Dough with longer relaxation times have been found to correlate with good baking properties since the dough is stronger (Dobraszyck & Morgenstern, 2003). Creep recovery experiments were carried out on dough prepared using the four cultivars and as a function of NaCl levels. A two-way analysis of variance of  $J_{\max}$  and of  $J_{el}$  found that both flour cultivar and NaCl level, along with their associated interaction were significant ( $p < 0.001$ ). Overall, during the creep phase, the amount of deformation of the dough ( $J_{\max}$ ) decreased with increasing NaCl levels indicating that the gluten network is becoming stronger as it is able to resist the imposed stress (Figure 4.3A). Harvest was observed to show the greatest  $J_{\max}$  suggesting that the dough was most susceptible to the imposed stress, followed by Roblin and McKenzie which were similar, and then Pembina. Dough prepared using Pembina showed the least amount of NaCl sensitivity, especially between the 1% and 2% levels (Figure 4.3A). With respect to the recovery phase, the relative elasticity of the doughs was found to increase with increasing NaCl levels for all flour cultivars. The magnitude of  $J_{el}$  was found to be greatest for Pembina, which had the strongest more elastic networks, followed by Roblin, McKenzie and then Harvest, which had the weakest gluten network (Figure 4.3B).

#### 4.4.2 Dough stickiness

Dough stickiness measurements are utilized to examine the adhesive forces (interactions between the dough and mixing surface) and the cohesive forces (interactions within the dough). Dough with high stickiness values indicates higher adhesive forces and lower cohesive forces (i.e., weak dough) whereas low stickiness values indicate lower adhesive forces and higher cohesive forces (i.e., strong dough). A two-way analysis of variance on dough stickiness values found the main effects of NaCl level and flour cultivar, along with their interaction were statistically significant ( $p < 0.001$ ). Overall between the 0 and 2% NaCl levels, Pembina and Roblin had the least stickiness relative to McKenzie and Harvest (Figure 4.4). Both Pembina and Roblin are considered to form strong gluten network, whereas McKenzie and Harvest have more intermediate and weak gluten strengths, respectively. In the case of Harvest, dough stickiness increased from 0.570 N at 2% NaCl to 0.689 N at 0% NaCl in relatively a linear fashion

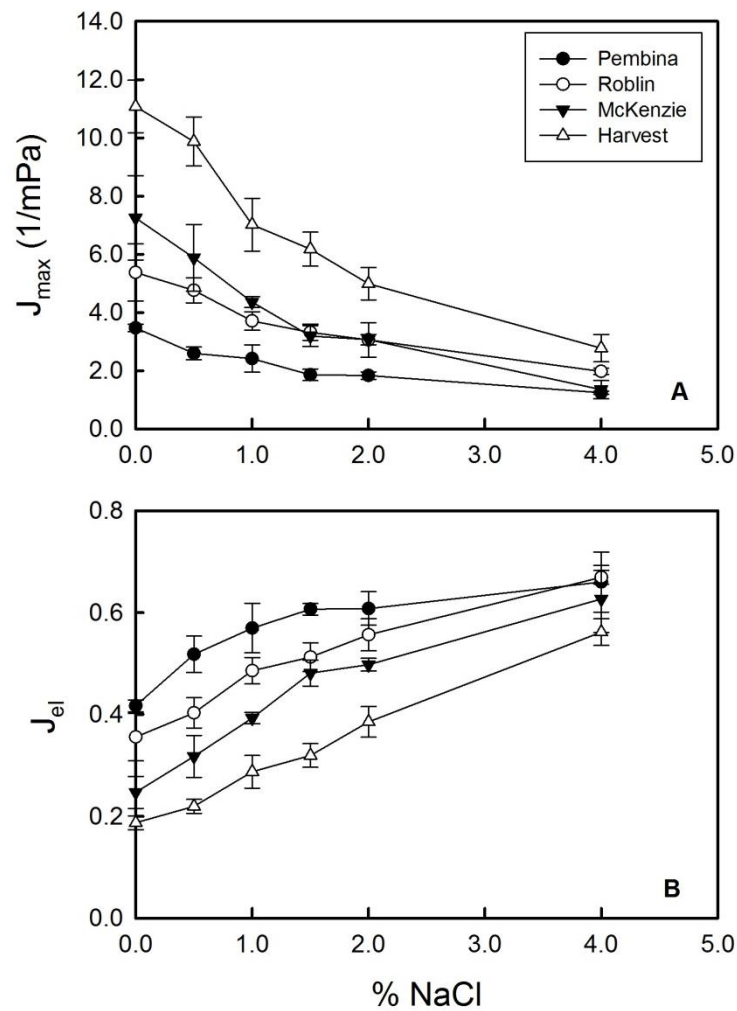


Figure 4.3 Maximum creep compliance ( $J_{max}$ ) (A) and relative elasticity ( $J_{el}$ ) (B) for dough prepared using different CWRS wheat cultivars (Pembina, Roblin, McKenzie and Harvest) as a function of NaCl level. Data represents the mean  $\pm$  one standard deviation ( $n = 3$ ).

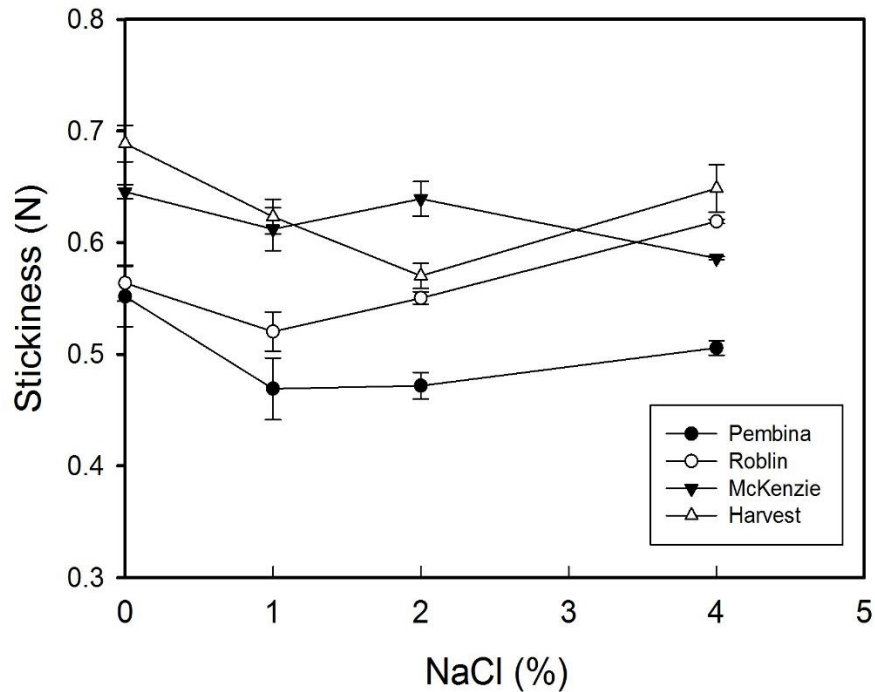


Figure 4.4 Stickiness values for dough prepared using different CWRs wheat cultivars (Pembina, Roblin, McKenzie and Harvest) as a function of NaCl level. Data represents the mean  $\pm$  one standard deviation ( $n = 3$ ).

( $p < 0.001$ ) (Figure 4.4). In contrast, McKenzie remained relatively constant with NaCl level between 0 and 2% NaCl with average stickiness values of 0.646 N and 0.639 N ( $p < 0.05$ ) (Figure 4.4). Pembina on the other hand was found to have similar stickiness values at the 1 and 2% NaCl ( $p > 0.05$ ), and then experienced an increase at the 0% NaCl ( $p < 0.001$ ). Stickiness for Roblin was found to decrease slightly from 0.550 N to 0.520 N as NaCl levels were lowered from 2% to 1% ( $p < 0.05$ ), and then increased to 0.564 N at the 0% NaCl ( $p < 0.01$ ) (Figure 4.4). Harvest, Roblin and Pembina saw an increase in stickiness values as NaCl levels were raised from 2% to 4%, where values increased from 0.570 N to 0.649 N ( $p < 0.001$ ), 0.550 N to 0.619 N ( $p < 0.001$ ), and 0.472 N to 0.506 ( $p < 0.05$ ), respectively. In contrast, for McKenzie a slight decrease in stickiness was observed where values were reduced from 0.639 N to 0.586 N as NaCl levels increased from 2% to 4% ( $p < 0.001$ ). From the stickiness results it can be seen that the degree to which each cultivar is impacted by NaCl reduction is cultivar dependent which most likely arises from differences in flour composition (gluten content/composition, starch

content/composition and non-starch polysaccharide content/composition) and quality as these compositional components have an impact on level of gluten protein hydration which impacts the level of protein-protein interactions.

#### **4.4.3 Dough extensibility**

Measuring the dough's resistance to extension and extensibility gives another indication of dough strength. There needs to be a balance between the dough's resistance to extension and extensibility, as this dictates gas pore expansion, for CO<sub>2</sub> retention to achieve the appropriate final loaf volume (Khatkar et al., 1995; Beck et al., 2012a; Delcour & Hoseney, 2013b). A two-way analysis of variance on dough resistance to extension values found the main effects of NaCl level and flour cultivar, along with their interaction were statistically significant ( $p < 0.001$ ). Overall, in Figure 4.5A, the trend was that with increasing NaCl levels there was an increase in resistance to extension although for Pembina this increase was more drastic at the 4% NaCl level. Pembina and Harvest were more affected by the 1% NaCl addition as their resistance to extension significantly increased for 0-1% NaCl whereas McKenzie and Roblin were not significantly affected by the 1% NaCl addition. All flour cultivars noticed a significant increase in their resistance to extension with the addition of 2-4% when compared to the 0% NaCl level ( $p < 0.01$ ). This increase in resistance to extension with the addition of NaCl is an indication of greater protein-protein interactions occurring as the NaCl shields the charges on the proteins and allows them to form a stronger gluten network through hydrophobic and hydrogen bonding to build up the gluten network. Pembina and Roblin consistently formed stronger gluten networks with greater resistance to extension followed by McKenzie and Harvest with the weakest gluten network formation at all NaCl levels.

A two-way analysis of variance on dough extensibility values found the main effects of NaCl level and flour cultivar, along with their interaction were statistically significant ( $p < 0.01$ ). In Figure 4.5B it can be seen that when comparing the doughs at the 0% NaCl level, Pembina appears to have the highest extensibility followed by Roblin and then Harvest however they are not significantly different ( $p > 0.05$ ). McKenzie has the lowest extensibility of all the dough cultivars at 0% NaCl ( $p < 0.05$ ). At 1% NaCl addition all flour cultivars except Harvest noticed a significant increase in extensibility ( $p < 0.001$ ) with the magnitude being cultivar dependent. Harvest didn't exhibit a significant increase in extensibility until the 2% NaCl addition ( $p < 0.001$ )

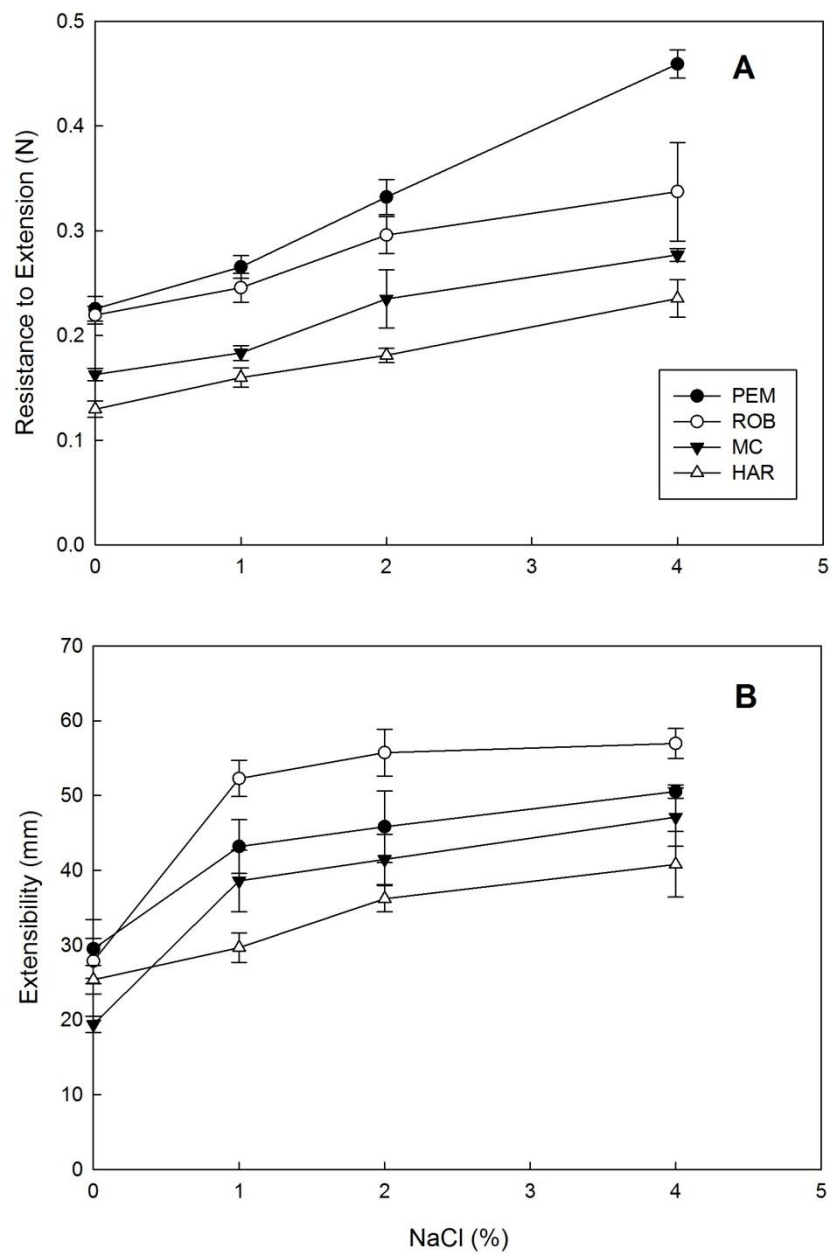


Figure 4.5 Resistance to extension (N) [A] and extensibility (mm) [B] values for dough prepared using different CWRS wheat cultivars (Pembina, Roblin, McKenzie and Harvest) as a function of NaCl level. Data represents the mean  $\pm$  one standard deviation ( $n = 3$ ).

further indicating a weaker flour that requires greater NaCl to induce strengthening effects. At the 2-4% level the extensibilities continued to increase only slightly from the 1% level.

#### **4.4.4 Freezable water content (FWC)**

Differential scanning calorimetry is commonly used to give an indication of how water is distributed in foods by distinguishing water that is freezable (free/unbound water) and water that is unfreezable (bound water) (Mak, 2009). A two-way analysis of variance of the freezable water content (FWC) of doughs and the main effects of flour cultivar and NaCl level were significant along with their two-way interaction ( $p < 0.001$ ). Overall, in Figure 4.6, Pembina was found to have the lowest freezable water content of all the cultivars. It should be noted that the doughs for each cultivar are made with different optimal water additions with Pembina having farinograph absorption of 61.9%, Roblin 65.0%, McKenzie 64.1% and Harvest having 65.5%. By adding the specific optimal water amounts to each flour cultivar it was believed that this would be a means of standardizing the doughs' performance. However in doing so, some of the observed differences within the FWC data may be attributed to this practice. That said, it was presumed to only have a minor effect on the FWC data and was more related to the strength of the gluten network, which is related to the gluten proteins within each flour cultivar and the NaCl levels. For instance, Roblin and Harvest both had similar amounts of water added during dough formation; however the FWC in dough prepared with Roblin flour was significantly lower than that of Harvest at the 1% NaCl level. In contrast at the 0% NaCl level, Roblin, McKenzie, and Harvest all have similar FWC whereas they all have slightly different water additions. Although, overall, there were slight differences found in response to the NaCl for all flours for FWC, no discernible trends were identified (Figure 4.6).

Since the effect of flour cultivar seems to dominate, the freezing effects of free and unbound water will be discussed for flour cultivar only. It was hypothesized that the stronger gluten network formed with Pembina would result in a greater amount of entrapped water (more strongly absorbed multilayer water) within the dough that would be less mobile (or free). In contrast, the weaker gluten network formed with Harvest is presumed to have a greater amount of mobile water that is freezable. In considering dough stickiness, this effect would cause the dough prepared with Harvest to become sticky, and Pembina not. It is interesting to note that at



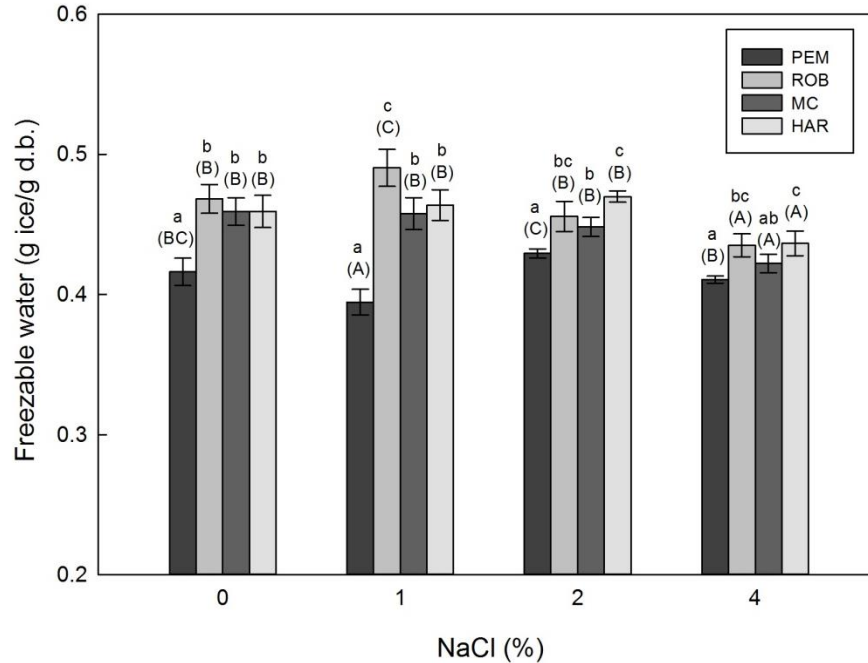


Figure 4.6 Freezable water content (g ice/g sample d.b.) values for dough prepared using different CWRs wheat cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC] and Harvest [HAR]) as a function of NaCl level. Data represents the mean  $\pm$  one standard deviation (n = 3). Upper case letters represent comparisons within a flour cultivar at each NaCl level. Lower case letters represent comparisons between flour cultivars at the same NaCl level. Different letters represent significant differences ( $p < 0.05$ ).

the 1% NaCl level for Roblin that it has the highest amount of FWC of the cultivars, however when looking at the stickiness results in Figure 4.4, it has the second lowest stickiness, with McKenzie and Harvest having higher stickiness values however they have lower FWC values. This may further point towards the hypothesis that there is a difference in the FWC location within the doughs and whether the water is entrapped (more strongly absorbed multilayer water, less mobile) within the gluten network formation (as it most likely is within the stronger cultivar of Roblin versus being near the surface of the dough in the diffusive water layer (weakly absorbed free water entrapped near the surface of the dough; more mobile) as it most likely is with McKenzie and Harvest which would most likely lead to the greater adhesive forces between the dough's surface and the stickiness probe). It is also interesting to note that in all cultivars except for Pembina, at the 4% NaCl level there is the lowest FWC when comparing NaCl levels

within each separate flour cultivar. And at the 1% NaCl level, a decrease in the FWC was observed for Pembina compared to the 0% level. In the case of Roblin, an increase in FWC was observed at the 1%NaCl level relative to the 0% level. McKenzie and Harvest have similar FWC for both 0% and 1% which are both weaker flours than Pembina and Roblin which may again further point to the dominance of effect of flour cultivar versus the effect of NaCl level.

#### **4.4.5 Thermogravimetric analysis (TGA)**

In a previous work by Fessas and Schiraldi (2001), thermogravimetric analysis (TGA) was utilized as an indicator of water being released from dough in two processes: the first through a diffusion process and second, by the desorption of the water that was more tightly bound to the gluten network. Therefore, TGA was applied in the present study to describe the different water associations with respect to either being near the surface of the dough existing as diffusive water (*which would relate more to the diffusion process*) or entrapped within the gluten network (*which would relate more to the more strongly absorbed multilayer and bound water*). Figure 4.7 gives a typical TGA scan seen for the dough samples used in this study. The first peak of the derivative gives an indication of water that is weakly interacting with the gluten network and is considered a part of the diffusive water layer. The second peak is in accordance with the water that is more strongly associated with the gluten network either entrapped within the network or bound by the gluten proteins. The effects of flour cultivar and NaCl level on weight loss and maximum peak height temperature for peak one and two are presented in Table 4.1. A two-way ANOVA found the effect of flour cultivar ( $p<0.001$ ) and NaCl level ( $p<0.01$ ) on weight loss of the first peak to be significant, along with their interaction ( $p<0.001$ ). Overall, in relation to the first peak weight loss when compared to other flour cultivars (regardless of NaCl level), Harvest (13.8%) showed the greatest amount of water loss ( $p<0.001$ ) followed by Roblin (10.5%), Pembina (9.7%) and McKenzie (8.4%). However, Pembina was not significantly different than Roblin and McKenzie ( $p>0.05$ ) but Roblin lost significantly more water than McKenzie ( $p<0.01$ ). These results are presumed to occur given that Harvest forms the weakest gluten network resulting in a greater amount of water near the surface of the dough (weakly absorbed free water near the surface; more mobile) instead of being entrapped (strongly absorbed) within the network whereas in the other flour cultivars, which form stronger gluten

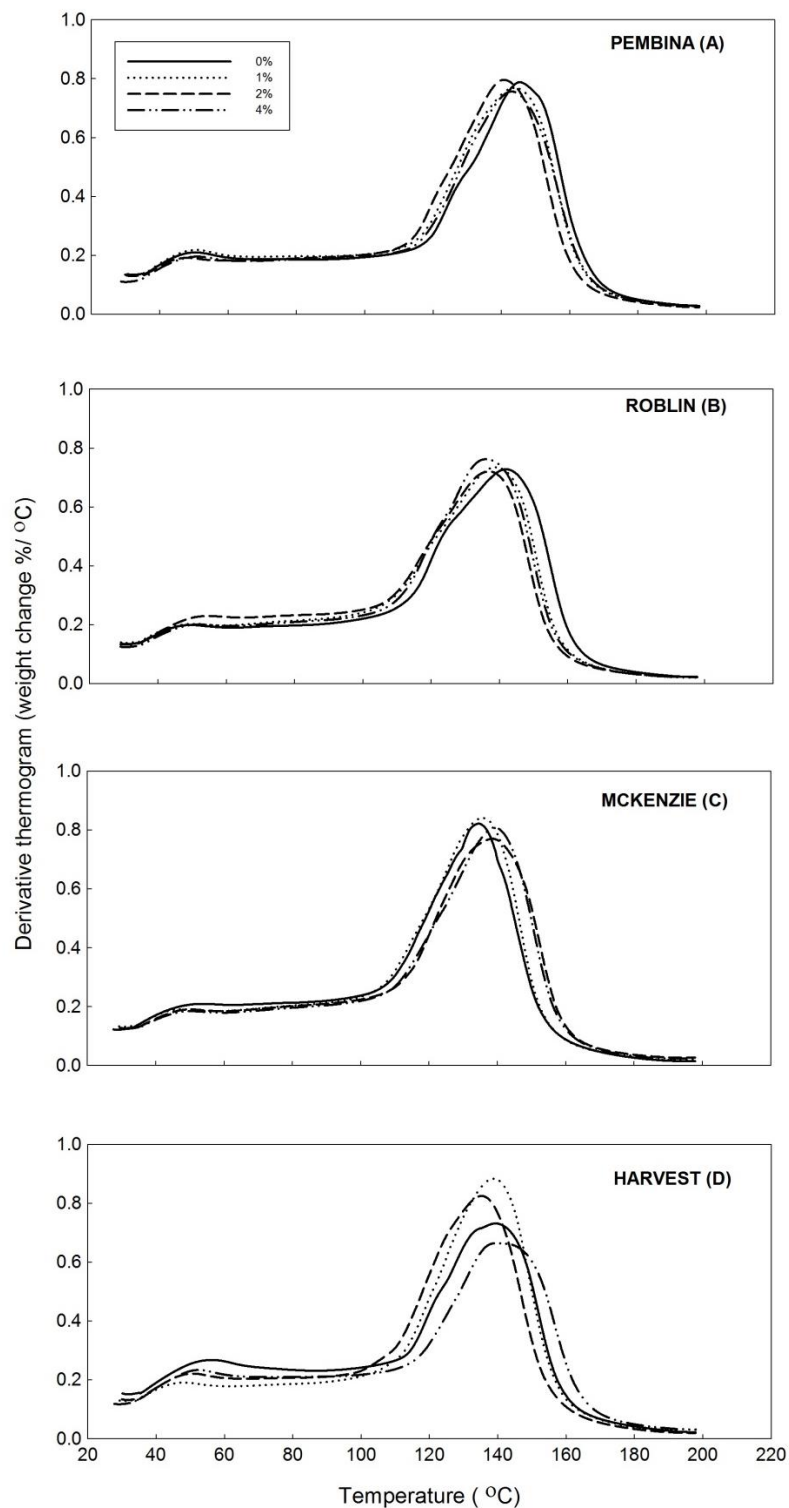


Figure 4.7 Derivative of the thermogram (weight change %/°C) of doughs prepared with CWRS wheat cultivars Pembina (A), Roblin (B), McKenzie (C), and Harvest (D) as a function of NaCl level.

Table 4.1 Thermogravimetric analysis values for dough prepared using CWRS wheat cultivars Pembina, Roblin, McKenzie and Harvest as a function of NaCl level. Data represents the mean  $\pm$  one standard deviation (n = 2).

Flour cultivar	NaCl (%)	1 <sup>st</sup> peak		2 <sup>nd</sup> peak		Total weight loss (%) <sup>2</sup>
		Max. peak height temp (°C)	Percent of total weight loss (%) <sup>1</sup>	Max. peak height temp (°C)	Percent of total weight loss (%) <sup>1</sup>	
Pembina	0	48.18 $\pm$ 3.01 <sup>a,A</sup>	9.49 $\pm$ 1.30 <sup>a,A</sup>	146.02 $\pm$ 1.05 <sup>c,C</sup>	63.97 $\pm$ 2.14 <sup>ab,AB</sup>	43.69 $\pm$ 0.05 <sup>bc,A</sup>
	1	50.80 $\pm$ 0.24 <sup>a,A</sup>	10.51 $\pm$ 0.66 <sup>a,B</sup>	144.54 $\pm$ 0.99 <sup>bc,C</sup>	61.89 $\pm$ 0.02 <sup>a,A</sup>	44.65 $\pm$ 0.12 <sup>c,AB</sup>
	2	49.18 $\pm$ 1.65 <sup>a,A</sup>	8.32 $\pm$ 0.04 <sup>a,A</sup>	139.91 $\pm$ 1.17 <sup>a,B</sup>	65.65 $\pm$ 1.99 <sup>b,B</sup>	42.61 $\pm$ 0.96 <sup>a,A</sup>
	4	50.97 $\pm$ 0.01 <sup>a,A</sup>	10.50 $\pm$ 0.88 <sup>a,A</sup>	142.51 $\pm$ 0.73 <sup>ab,B</sup>	66.94 $\pm$ 0.44 <sup>b,C</sup>	42.80 $\pm$ 0.14 <sup>ab,A</sup>
Roblin	0	51.4 $\pm$ 2.7 <sup>a,AB</sup>	10.5 $\pm$ 1.7 <sup>ab,A</sup>	142.5 $\pm$ 0.9 <sup>b,B</sup>	66.5 $\pm$ 2.8 <sup>b,B</sup>	44.0 $\pm$ 0.1 <sup>b,A</sup>
	1	49.6 $\pm$ 0.5 <sup>a,A</sup>	10.3 $\pm$ 0.5 <sup>ab,B</sup>	138.2 $\pm$ 0.8 <sup>a,AB</sup>	66.5 $\pm$ 0.2 <sup>b,BC</sup>	43.8 $\pm$ 0.2 <sup>ab,A</sup>
	2	55.2 $\pm$ 0.4 <sup>b,B</sup>	12.2 $\pm$ 1.1 <sup>b,B</sup>	137.7 $\pm$ 0.9 <sup>a,AB</sup>	61.5 $\pm$ 0.9 <sup>a,A</sup>	43.4 $\pm$ 0.3 <sup>ab,AB</sup>
	4	52.2 $\pm$ 3.8 <sup>ab,A</sup>	9.1 $\pm$ 2.0 <sup>a,A</sup>	140.1 $\pm$ 1.3 <sup>ab,AB</sup>	64.6 $\pm$ 0.5 <sup>ab,BC</sup>	43.0 $\pm$ 0.3 <sup>a,A</sup>
McKenzie	0	52.4 $\pm$ 1.9 <sup>b,B</sup>	9.0 $\pm$ 0.6 <sup>a,A</sup>	136.9 $\pm$ 3.2 <sup>ab,A</sup>	64.1 $\pm$ 0.9 <sup>a,B</sup>	43.8 $\pm$ 1.0 <sup>a,A</sup>
	1	47.8 $\pm$ 0.0 <sup>a,A</sup>	7.0 $\pm$ 0.2 <sup>a,A</sup>	135.4 $\pm$ 0.3 <sup>a,A</sup>	65.6 $\pm$ 0.0 <sup>a,AB</sup>	43.9 $\pm$ 0.3 <sup>a,A</sup>
	2	50.0 $\pm$ 0.5 <sup>ab,A</sup>	8.7 $\pm$ 0.9 <sup>a,A</sup>	137.2 $\pm$ 2.0 <sup>ab,AB</sup>	64.4 $\pm$ 0.6 <sup>a,AB</sup>	43.7 $\pm$ 0.2 <sup>a,B</sup>
	4	50.7 $\pm$ 0.5 <sup>ab,A</sup>	9.0 $\pm$ 0.1 <sup>a,A</sup>	138.7 $\pm$ 0.7 <sup>b,A</sup>	63.1 $\pm$ 2.5 <sup>a,B</sup>	43.1 $\pm$ 0.1 <sup>a,A</sup>

Table 4.1 (cont.)

Flour cultivar	NaCl (%)	1 <sup>st</sup> peak		2 <sup>nd</sup> peak		Total weight loss (%) <sup>2</sup>
		Max. peak height temp (°C)	Percent of total weight loss (%) <sup>1</sup>	Max. peak height temp (°C)	Percent of total weight loss (%) <sup>1</sup>	
Harvest	0	56.75 ± 0.49 <sup>b,C</sup>	19.80 ± 2.19 <sup>c,B</sup>	141.35 ± 2.80 <sup>b,B</sup>	62.23 ± 0.11 <sup>b,A</sup>	46.68 ± 0.91 <sup>c,B</sup>
	1	48.56 ± 0.63 <sup>a,A</sup>	9.07 ± 1.55 <sup>a,AB</sup>	138.99 ± 0.31 <sup>ab,B</sup>	69.31 ± 4.29 <sup>c,C</sup>	45.40 ± 0.22 <sup>b,B</sup>
	2	49.05 ± 2.35 <sup>a,A</sup>	9.68 ± 1.61 <sup>a,AB</sup>	136.21 ± 1.35 <sup>a,A</sup>	68.00 ± 1.35 <sup>c,B</sup>	45.00 ± 0.00 <sup>b,C</sup>
	4	54.08 ± 2.39 <sup>b,A</sup>	16.80 ± 1.56 <sup>b,B</sup>	140.54 ± 1.42 <sup>b,AB</sup>	58.26 ± 1.52 <sup>a,A</sup>	43.75 ± 0.30 <sup>a,A</sup>

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Different letters for each flour cultivar in each column are significantly different ( $p < 0.05$ ). The lower case letters are comparisons within a cultivar and upper case letters are comparisons between cultivars at each NaCl level.

<sup>1</sup>Percent of total weight loss % at the first and second peak was calculated by over laying the thermogram of weight % curve over the derivative weight change (weight change % /°C) curve and subtracting the end limit of the peak by the onset limit of the peak to obtain weight loss % over the peak interval. This value was then divided by the total weight loss % and multiplied by 100 to get the percent of total weight loss.

<sup>2</sup>Total weight loss (%) was calculated by: [(initial weight mg – final weight mg)/ initial weight mg] x 100.

networks are believed to entrap more water within the gluten network and contain less weakly absorbed free water near the surface of the dough.

The effect of NaCl was different in the case of each flour cultivar. In the case of Pembina there appears to be no apparent trend with increasing NaCl level with weight loss ranging from (8.3-10.5%). In the case of Roblin there again doesn't appear to be an apparent trend except for the fact that 2% NaCl lost a greater amount of weight (12.2%) than the 4% NaCl level (9.1%). This result is similar to the findings for Roblin in the FWC with 2% having a greater level of FWC than 4% (Figure 4.6). However, this does not coincide with the findings from dough stickiness where stickiness is seen to increase when going from 2-4% (Figure 4.4). In the case of McKenzie again there appears to be no apparent trend with weight loss ranging from (7.0-9.0%) which lost the least amount of water at the first peak relating to the diffusive water layer. In the case of Harvest, it appears to be the most affected by the changing NaCl levels, losing the greatest amount of water at 0% (19.8%) and 4% (16.8%) whereas the amount of water lost at the 1 and 2% NaCl level was more similar to that of the other flours. This could be an indication of a change in the water's spatial distribution within the dough from near the surface or free water weakly absorbed to having a greater amount becoming entrapped (more strongly absorbed) as a result of the NaCl resulting in a greater formed gluten network. In contrast, for Harvest, weight loss at the first peak declined from ~20% with 0% NaCl to ~11% in the presence of NaCl (in the range of 1-2%), however at the 4% level Harvest exhibited an increase ~17% (Table 4.1). The greater loss at the 0% NaCl level is thought to be associated with a weaker gluten network being formed than in the presence of NaCl, allowing for greater free water weakly absorbed near the surface. This hypothesis is supported by the stickiness results, where stickiness was greatest at the 0 and 4% NaCl level and lower from 1 to 2%.

A two-way ANOVA found the first peak temperature to be impacted by flour cultivar ( $p < 0.05$ ) and NaCl level ( $p < 0.05$ ) along with their interaction ( $p < 0.01$ ). Overall, regardless of NaCl level, Pembina experienced a lower temperature (49.8°C) required to release the diffusive water than Roblin (52.1°C) and Harvest (52.1°C) ( $p < 0.05$ ) however it was similar to McKenzie (50.2°C) ( $p > 0.05$ ). The temperatures required to release the diffusive water from Roblin, McKenzie and Harvest were found to be similar as well ( $p > 0.05$ ). This was supported by DSC data (Figure 4.6), it can be seen that Pembina (Figure 4.7A), was less affected by NaCl levels than the other flours (Figure 4.7B-D).

A two-way ANOVA of the second peak weight loss found the effect of NaCl level and flour cultivar to not be significant ( $p>0.05$ ), however their interaction was highly significant ( $p<0.001$ ). Table 4.1 shows no obvious trends in NaCl levels within each flour cultivar, with the exception of Harvest where at the 1 and 2% NaCl levels the greatest amount of weight is lost in the second peak and the least amount is lost in the first peak and vice versa for the 0 and 4% NaCl levels. This again follows along with the stickiness trend seen for the stickiness data. When comparing the different flour cultivars at each NaCl level, at the 0% level only Roblin and Harvest were significantly different with Roblin losing a greater amount (66.5%) than Harvest (62.2%).

A two-way ANOVA found the second max peak height temperature to show significant effects of flour cultivar ( $p<0.001$ ) and NaCl level ( $p<0.001$ ), however, their interaction was not significant ( $p>0.05$ ). Overall when comparing flour cultivars, regardless of NaCl level, Pembina had the highest second peak max temperature (143.2°C) ( $p<0.001$ ) followed by Roblin (139.6°C) and Harvest (139.3°C) which were similar ( $p>0.05$ ), and McKenzie which has the lowest temperature (137.0°C) ( $p<0.01$ ). Overall when comparing NaCl level regardless of flour cultivar, the trend seems to be that at the 2% NaCl level there is a decrease in the peak temperature (137.8°C) whereas at 0, 1 and 4% NaCl they have fairly similar but slightly higher temperatures of 141.7°C, 139.3°C, and 140.5°C respectively. Peak temperature for 2% NaCl is significantly lower than both 0% and 4% ( $p<0.01$ ), however it is similar to the 1% temperature ( $p>0.05$ ). The second peak temperature for no salt addition is significantly higher than that of 1 and 2% NaCl. It was hypothesized this was occurring because at 0% NaCl level, more of the water is hydrating the gluten proteins and therefore it takes more energy to pull off, whereas with the inclusion of NaCl there is less hydration of the proteins due to increased protein-protein interaction and increased water-ion interaction.

With respect to total weight loss a two-way ANOVA shows that the main effects of NaCl ( $p<0.001$ ) and flour cultivar ( $p<0.001$ ) are significant as well as their interaction ( $p<0.05$ ). The overall trend when considering the effect of NaCl, regardless of flour cultivar, is with increasing amounts of NaCl there is a slight decrease in the amount of water lost with each level of NaCl ( $p<0.05$ ) (Table 4.1). However, 0% and 1% NaCl total weight loss is not significantly different ( $p>0.05$ ). When considering the effect of flour cultivar, regardless of NaCl level, the trend is that Harvest loses the greatest amount of water at 45.2% ( $p<0.001$ ) whereas Pembina, Roblin and

McKenzie had similar total weight losses of ~43.6% ( $p>0.05$ ). It is interesting to note that at the 4% NaCl level that there is no significant difference in total weight loss for any of the cultivars, whereas at 0% Harvest lost the highest amount and, Pembina, Roblin and McKenzie lost similar amounts of water. Findings suggest that the effect of NaCl is cultivar specific, with certain cultivars being more sensitive than others. At the 1% level Pembina has a similar total weight loss as the other cultivars of Roblin, McKenzie and Harvest. However, Roblin and McKenzie have slightly lower total weight loss than Harvest ( $p<0.01$ ) but are similar in magnitude to each other ( $p>0.05$ ). At the 2% NaCl level it can be seen that Pembina has the lowest total weight loss when compared to Harvest ( $p<0.001$ ) and McKenzie ( $p<0.05$ ) but a similar weight loss to Roblin ( $p>0.05$ ) which is interesting as these two cultivars were found to be the strongest at the 2% level in previous studies. Within each flour cultivar a common trend is that with increasing NaCl level there is a slight decrease in the total weight loss.

#### *4.4.5.1 Thermogravimetric analysis (Deconvolution of the derivative thermogram DTG)*

Fessas and Schiraldi (2001) stated that in freshly mixed dough, water is shared among its different components (e.g., starches, proteins, etc.) and also within interphase regions (i.e., free water). And as such, a deconvolution of the TGA derivative thermogram can be used to help discern which component/phase the water is associated with. In the present study, the algorithm was performed according to Roozendaal et al. (2012), and identified water as free water, or water associated with the starch or gluten components. A typical derivative thermogram is given in Figure 4.8A for dough prepared from Pembina at 0% NaCl, along with its associated deconvolution in Figure 4.8B. Although a 5 peak model was used in Roozendaal et al. (2012), a 6 peak model was used in this study as the 6 peak model produced a higher agreement with the DTG  $r^2$  fit. The peaks represent either free water (peak 1 and 2), mobile/unbound and bound water to the starch (peak 3 and 4, respectively), or mobile/unbound and bound water to the gluten proteins (peak 5 and 6, respectively). Other curves appeared similar for other flour cultivars and NaCl levels, only peaks differed in magnitude.

A two-way analysis of variance of peak temperatures found that the main effects of NaCl level and flour cultivar, along with their associated interaction were highly significant ( $p<0.001$ ) for both free water (peaks 1 and 2) and starch-fraction (peaks 3 and 4) peaks. In the case of peak



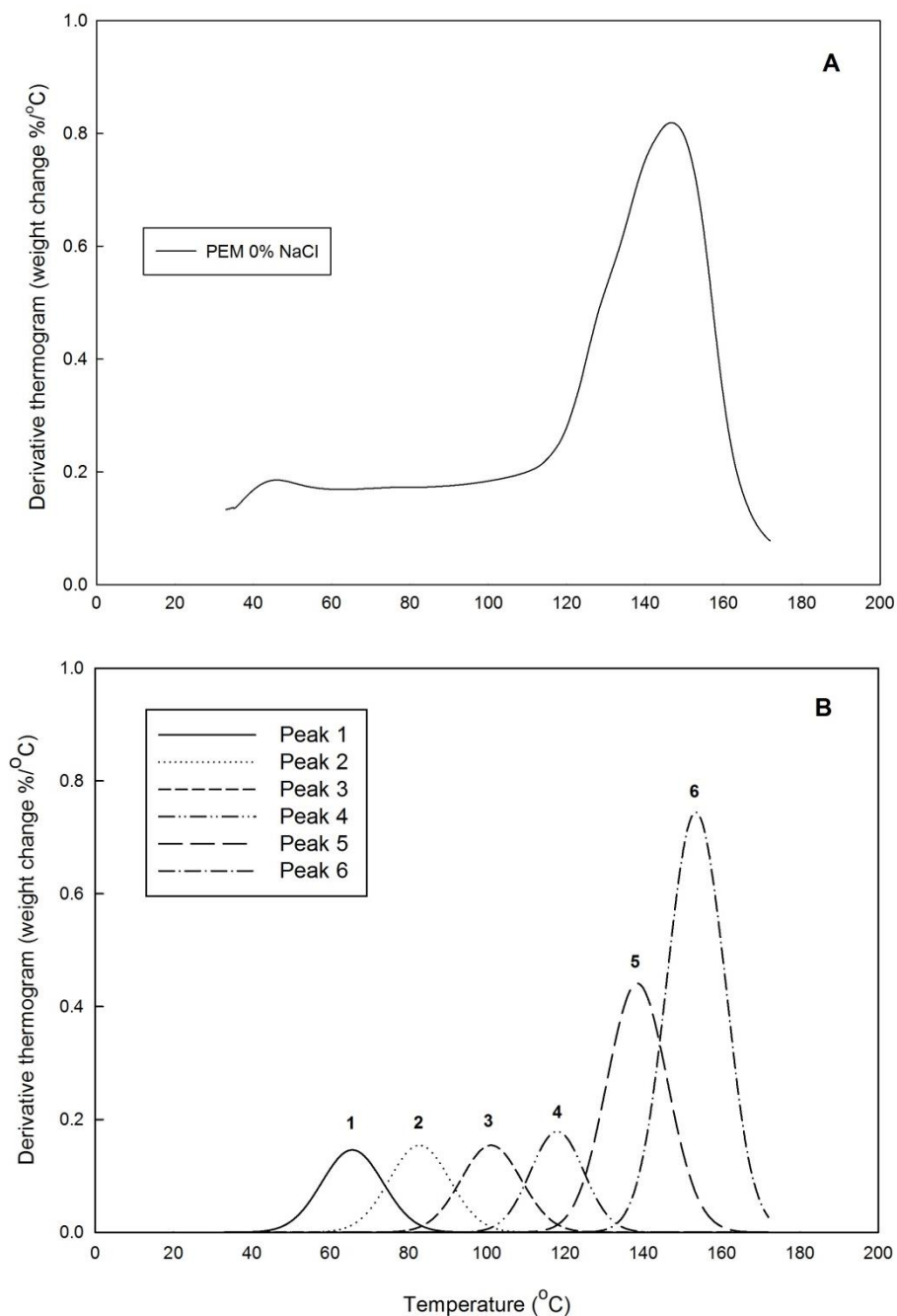


Figure 4.8 Derivative thermogram (weight change %/°C) for a dough prepared with Pembina at 0% NaCl (A) and the deconvolution of the derivative thermogram (B). Peak 1 & 2 represents water associated with free water, peak 3 & 4 represents water associated with starch both mobile and bound respectively, and peak 5 & 6 represent water associated with gluten both mobile and bound respectively.

temperature associated with the gluten-fraction, the effect of NaCl level and flour cultivar were significant ( $p < 0.01$ ), whereas the interaction between the main effects was not ( $p > 0.05$ ). Within Table 4.2 the overall trend for peak temperatures, when comparing between NaCl levels within flour cultivar, showed a decrease in temperatures required to remove the water for each peak with the addition of NaCl. It is interesting to note that Pembina requires the highest temperature ( $\sim 149^\circ\text{C}$ ) to pull the water from the bound gluten peak (peak 6) at the 0% NaCl relative to Roblin, McKenzie and Harvest which were all fairly similar. This may correspond to a more ordered gluten network that is able to entrap water better (absorb more strongly), resulting in a stronger and non-sticky dough. With the addition of 2% NaCl it can be seen that the peak temperature for water loss of bound gluten water (peak 6) is similar among the flour cultivars ( $\sim 140^\circ\text{C}$ ). The latter, may suggest why flours perform well at the 2% level in terms of dough handling.

Overall, when comparing the 0% to 2% NaCl within each flour cultivar, the water loss for the unbound and bound starch (peaks 3 and 4) and gluten (peaks 5 and 6) do not differ greatly. However there is a notable change in the water loss for the free water peaks (peaks 1 and 2). The greatest changes are seen within Pembina and Harvest, with both experiencing a decrease in the amount of free water lost in peaks 1 and 2 with the addition of 2% NaCl. Based on these findings further discussions regarding water loss will be discussed as it relates to free water (peak 1 + 2), the starch-fraction (peak 3 + 4) and the gluten-fraction (peak 5 + 6), as shown in Figure 4.9. The corresponding two-way ANOVA results are given in Table 4.3. For free water, the main effects of NaCl level ( $p < 0.001$ ) and flour cultivar ( $p < 0.01$ ) were significant, along with their associated interaction ( $p < 0.01$ ). The overall trend when comparing NaCl level, regardless of flour cultivar, was that with the removal of NaCl there was an increase in the amount of free water loss from  $\sim 15\%$  with 2% NaCl to  $\sim 21\%$  with 0% NaCl ( $p < 0.001$ ). At the 0% NaCl level, Pembina and McKenzie showed the lowest amount of free water loss ( $\sim 18\%$ ) followed by Roblin ( $\sim 22\%$ ) and Harvest ( $\sim 28\%$ ) with the highest. However, at the 2% NaCl there was no significant difference between the free water loss between each flour cultivar.

A two-way ANOVA of the peak water loss associated with the starch-fraction found that the main effects of NaCl level ( $p < 0.01$ ) and flour cultivar ( $p < 0.05$ ) were significant, along with their associated interaction ( $p < 0.05$ ). The overall trend, when comparing NaCl level regardless of flour cultivar, is that more water was lost from the starch-fraction at the 2% NaCl level ( $\sim 22\%$ )

Table 4.2 Peak fitting data for peak temperature and relative proportion of peak water loss for doughs prepared using CWRs wheat cultivars (Pembina, Roblin, McKenzie, Harvest) as a function of 0% and 2% NaCl. Values represent mean samples  $\pm$  one standard deviation (n=2).

Flour Cultivar	Water's Association	Peak Number	Peak Temperature ( $^{\circ}$ C)		Relative Proportion of Peak Water Loss (%)	
			0% NaCl	2% NaCl	0% NaCl	2% NaCl
Pembina	Free	1	41.1 $\pm$ 0.6	42.4 $\pm$ 0.3	8.7 $\pm$ 0.4	7.4 $\pm$ 0.1
		2	61.7 $\pm$ 0.2	58.8 $\pm$ 0.2	9.4 $\pm$ 0.8	6.9 $\pm$ 0.3
	Starch	3	84.2 $\pm$ 0.2	81.4 $\pm$ 0.6	8.9 $\pm$ 0.1	12.7 $\pm$ 0.2
		4	105.5 $\pm$ 0.3	102.4 $\pm$ 0.3	10.4 $\pm$ 2.0	8.9 $\pm$ 1.3
	Gluten	5	131.5 $\pm$ 1.3	124.8 $\pm$ 0.7	25.8 $\pm$ 0.8	22.2 $\pm$ 2.7
		6	149.2 $\pm$ 0.5	141.8 $\pm$ 0.2	36.8 $\pm$ 4.2	41.8 $\pm$ 1.9
Roblin	Free	1	46.5 $\pm$ 0.3	41.9 $\pm$ 0.3	11.3 $\pm$ 1.1	7.6 $\pm$ 2.3
		2	68.9 $\pm$ 0.2	53.7 $\pm$ 0.9	10.4 $\pm$ 0.0	7.7 $\pm$ 0.4
	Starch	3	90.1 $\pm$ 1.1	74.4 $\pm$ 0.9	10.5 $\pm$ 0.9	12.4 $\pm$ 0.5
		4	103.4 $\pm$ 0.6	96.5 $\pm$ 0.2	6.4 $\pm$ 1.9	13.0 $\pm$ 1.7
	Gluten	5	124.1 $\pm$ 0.7	122.2 $\pm$ 2.1	23.0 $\pm$ 0.2	24.3 $\pm$ 1.1
		6	144.2 $\pm$ 0.2	140.4 $\pm$ 1.4	38.4 $\pm$ 2.4	35.1 $\pm$ 0.4
McKenzie	Free	1	42.5 $\pm$ 0.4	39.5 $\pm$ 0.2	8.5 $\pm$ 1.3	7.4 $\pm$ 0.7
		2	61.2 $\pm$ 0.7	57.1 $\pm$ 0.2	9.5 $\pm$ 0.7	9.0 $\pm$ 0.0
	Starch	3	80.4 $\pm$ 0.1	77.5 $\pm$ 0.3	8.8 $\pm$ 0.5	10.4 $\pm$ 1.4
		4	98.8 $\pm$ 0.3	100.2 $\pm$ 0.9	9.5 $\pm$ 0.8	12.3 $\pm$ 1.6
	Gluten	5	121.8 $\pm$ 0.9	122.4 $\pm$ 2.9	23.2 $\pm$ 2.9	17.7 $\pm$ 3.8
		6	139.0 $\pm$ 2.2	138.9 $\pm$ 1.8	40.5 $\pm$ 5.2	43.2 $\pm$ 4.4
Harvest	Free	1	49.2 $\pm$ 0.8	41.5 $\pm$ 0.4	16.0 $\pm$ 2.0	6.5 $\pm$ 0.2
		2	72.9 $\pm$ 0.0	54.6 $\pm$ 0.7	11.6 $\pm$ 2.8	8.0 $\pm$ 0.1
	Starch	3	88.8 $\pm$ 0.3	74.6 $\pm$ 0.0	4.4 $\pm$ 2.4	9.2 $\pm$ 0.1
		4	102.9 $\pm$ 0.1	96.1 $\pm$ 0.4	10.8 $\pm$ 1.1	11.1 $\pm$ 1.3
	Gluten	5	126.2 $\pm$ 2.1	121.4 $\pm$ 0.3	22.6 $\pm$ 4.2	25.3 $\pm$ 1.1
		6	144.0 $\pm$ 2.8	139.0 $\pm$ 0.7	34.7 $\pm$ 3.7	39.9 $\pm$ 2.5

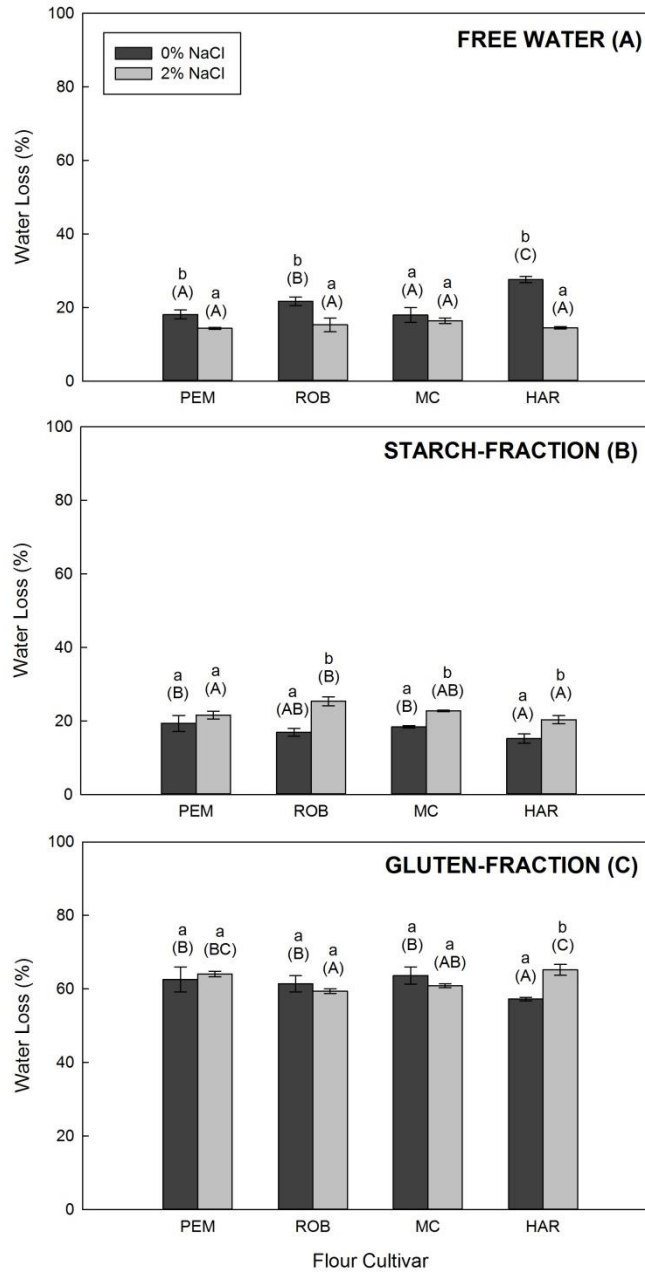


Figure 4.9 Peak fitting data representing total peak free water (A), starch-fraction (B), and gluten-fraction (C) water loss (%) for doughs prepared using CWRS wheat cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC], and Harvest [HAR]) as a function of 0% and 2% NaCl. Values represent means  $\pm$  sd. (n=2). Lower case letters represent comparisons within a flour cultivar between NaCl level. Upper case letters represent comparisons between flour cultivars at each NaCl level. Different levels are significantly different  $p < 0.05$ .

Table 4.3 Probability values arising from a two-way analysis of variance for peak fitting data for proportional area under the curve for free water peaks, starch water peaks and gluten water peaks for doughs prepared using CWRS wheat cultivars (Pembina, Roblin, McKenzie, Harvest) as a function of 0% and 2% NaCl.

	Peak area		
	Free water (Peaks 1 + 2)	Starch-fraction (Peaks 3 + 4)	Gluten-fraction (Peaks 5 + 6)
<b>Main effects</b>			
NaCl level	p < 0.001	p < 0.001	NS
Flour cultivar	p < 0.01	p < 0.05	NS
<b>Interaction</b>			
NaCl level*flour cultivar	p < 0.01	p < 0.05	p < 0.05

versus the 0% NaCl level (~17%). This trend is opposite to that of the free water loss where there was a decrease in water loss when going from 0% NaCl to 2% NaCl ( $p < 0.001$ ). It is hypothesized that at the 0% NaCl the doughs had more water present as free water than in the starch fraction, whereas at the 2% NaCl level water is more associated with the starch, and less so in free water form. When comparing flour cultivar, regardless of NaCl level, water loss for Pembina, Roblin and McKenzie are all similar (~21%) ( $p > 0.05$ ), whereas Harvest lost the least amount of water from the starch-fraction (~18%) ( $p < 0.05$ ). At the 0% NaCl level Harvest lost the least amount of water (~15%) compared to Pembina and McKenzie (~19%) ( $p < 0.05$ ). Roblin's water loss for the starch fraction was not significantly different from the other flours at 0% NaCl. When comparing flour cultivars at the 2% NaCl level there is a shift in amount of water lost. Harvest has an increase in water loss but still has the lowest amount of water lost (~20%), however is similar to Pembina (~22%) and McKenzie (~23%) ( $p > 0.05$ ) whereas Roblin has the highest amount of water lost (~25%) ( $p < 0.05$ ) but it is not significantly different from McKenzie.

A two-way ANOVA of the water loss associated with the gluten-fraction found that the main effects of NaCl level and flour cultivar were not significant ( $p > 0.05$ ), however their associated interaction was significant ( $p < 0.05$ ). When comparing flour cultivar at the 0% NaCl

level, Harvest had the lowest amount of water lost from the gluten-fraction (~57%) ( $p < 0.05$ ) followed by Roblin (~61%), Pembina (~63%) and McKenzie (~64%), which were not significantly different ( $p > 0.05$ ). For the 2% NaCl level there was no apparent trend observed between the different cultivars; Roblin lost the least amount of water (~59%) ( $p < 0.05$ ) followed by McKenzie (~61%) and Pembina (~64%), which were not significantly different, and Harvest lost the greatest amount for water from the gluten-fraction ( $p < 0.05$ ) however it is not significantly different from Pembina.

Based on the TGA findings, it was hypothesized that the gluten network may not be the only factor governing dough stickiness in reduced sodium environments, but rather the relationship between free water and that associated with the starch-fraction. The amount of water associated with the starch fraction increases at the 2% NaCl level whereas the amount of water associated with free water decreases. In contrast, at the 0% NaCl level the amount of water associated with the starch-fraction is decreased and the amount of free water is increased. Therefore, if the amount of water associated or absorbed by the starch at low NaCl levels could be increased this may reduce the dough stickiness problem in low sodium environments. A potential way to increase starch water absorption at low sodium levels would be to increase the damaged starch. It is further proposed that the strength of the gluten network would mask the change in water's association with the starch, which is why Pembina shows less stickiness at reduced sodium levels than Harvest which has a weaker gluten network formed.

#### **4.4.6 Confocal laser scanning microscopy (CLSM)**

Confocal laser scanning micrographs for doughs prepared from Pembina, Roblin, McKenzie and Harvest flours in the absence and presence of 2% NaCl are given in Figure 4.10. It was observed that doughs prepared in the absence of NaCl, for all cultivars, were more porous than those prepared at the 2% NaCl level. For doughs prepared from Pembina, Roblin and McKenzie flours, gluten proteins showed more directional orientation than without NaCl, hypothesized to lead to greater elastic properties. However, in the case of doughs prepared with Harvest flour (the weakest cultivar examined), less differences were observed between the 0 and 2% NaCl levels, with both displaying porous multi-directional orientation of the gluten polymers.

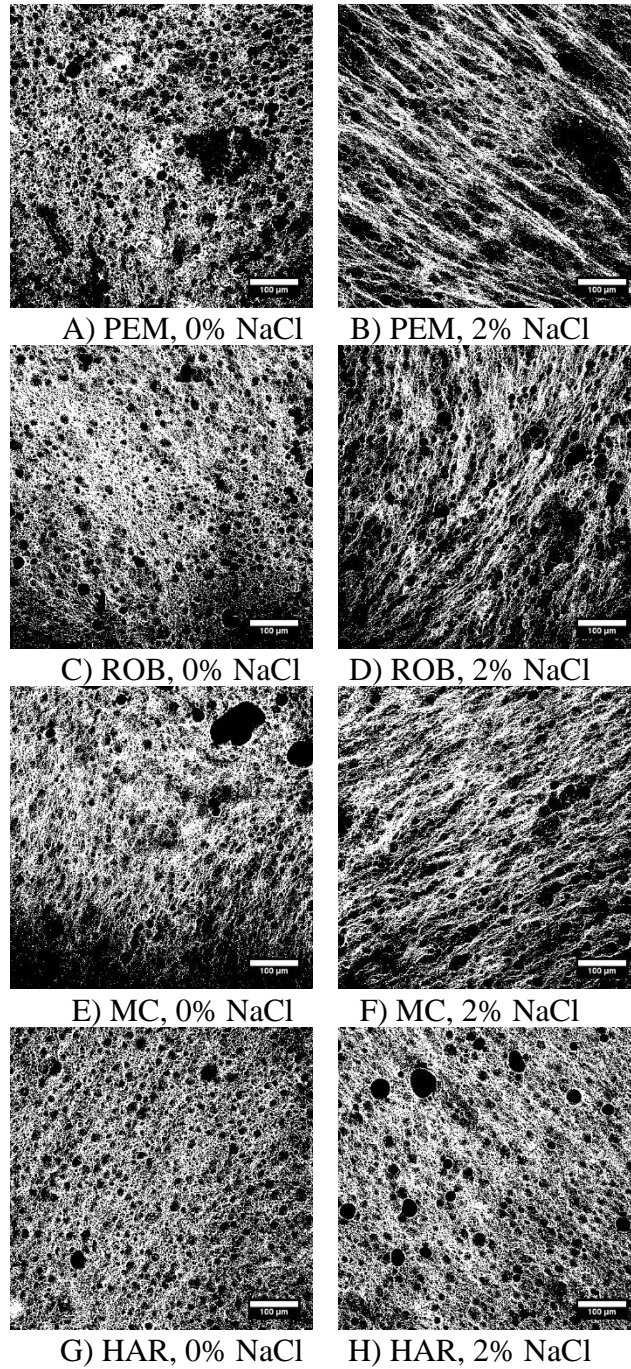


Figure 4.10 Confocal laser scanning microscopy images for dough prepared using different CWRS wheat cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC] and Harvest [HAR]) with 0% and 2% NaCl.

Doughs prepared with Pembina were found to be more elastic than the other doughs, hypothesized due to the greater organization of gluten polymers. Furthermore, stickiness was found to be reduced as NaCl levels increased between 0% and 2% for all flours (Figure 4.4), which is presumed to correlate with the transition from a porous, multi-directional oriented network of gluten polymers to one that is less porous, with longer unidirectional fibres. The less ordered porous network is thought to have a greater amount of adhesive forces present than cohesive forces leading to the higher stickiness values. Jekle and Becker (2011) noted that fewer protein interactions would lead to more mobile proteins resulting in a weakening effect on the dough and thus greater stickiness. In the current study, it is presumed that the doughs with more ordered morphology have a greater amount of protein-protein interactions and cohesive forces present leading to less sticky dough.

To give a quantitative analysis of the dough morphologies for doughs prepared with different flour cultivars without and with NaCl, image analysis was evaluated similar to Jekle and Becker (2011) and given in Table 4.4. In the present study particle count is associated with degree of protein aggregation. A lower particle count indicates a higher level of gluten protein aggregation and a weaker gluten network formation due to decreased gluten polymer interaction. On the other hand a higher particle count would indicate less gluten protein aggregation and an increased gluten network formation through increased gluten polymer strand interactions which are identifiable as separate particles from one another. A two-way analysis of variance was run on all image analysis parameters. For particle count the main effect of both flour cultivar and NaCl level were significant ( $p < 0.001$ ) however their interaction was not ( $p > 0.05$ ). When comparing particle count of the doughs prepared with different cultivars and NaCl level it can be seen that in all cultivars, except for Harvest, the particle counts significantly increase from 0% to 2% NaCl addition indicating a decrease in gluten protein aggregation and an increase in gluten polymer interaction/organization building up the gluten network. Findings suggest that Pembina continues to have the highest particle counts (least protein aggregation and thus greater gluten network formation) at both the 0% and 2% NaCl level; however results do overlap with McKenzie. Roblin and Harvest particle count are however significantly similar which is interesting given that Roblin appears to have a more directional gluten network formation as opposed to Harvest.



Table 4.4 Image analysis of confocal laser scanning microscopy of doughs prepared using CWRs wheat cultivars (Pembina [PEM], Roblin [ROB], McKenzie [MC], Harvest [HAR]) as a function of 0% and 2% NaCl. Values represent mean of 3 images  $\pm$  one standard deviations.

Flour cultivar	NaCl (%)	Particle Count	Total Particle Area ( $10^4 \mu\text{m}^2$ )	Average Particle Size ( $\mu\text{m}^2$ )	Circularity	Fractal Dimension
PEM	0	$4290 \pm 745^{\text{b},\text{A}}$	$12.80 \pm 2.94^{\text{a},\text{A}}$	$31.21 \pm 11.85^{\text{a},\text{A}}$	$0.89 \pm 0.01$	$1.85 \pm 0.05^{\text{a},\text{A}}$
	2	$5203 \pm 354^{\text{c},\text{B}}$	$15.83 \pm 1.80^{\text{b},\text{B}}$	$30.60 \pm 4.78^{\text{ab},\text{A}}$	$0.91 \pm 0.02$	$1.89 \pm 0.01^{\text{a},\text{A}}$
ROB	0	$2878 \pm 228^{\text{a},\text{A}}$	$19.53 \pm 0.73^{\text{c},\text{A}}$	$68.06 \pm 4.75^{\text{b},\text{B}}$	$0.89 \pm 0.01$	$1.93 \pm 0.01^{\text{b},\text{A}}$
	2	$4267 \pm 756^{\text{ab},\text{B}}$	$15.34 \pm 1.87^{\text{b},\text{B}}$	$37.23 \pm 10.90^{\text{bc},\text{A}}$	$0.89 \pm 0.01$	$1.90 \pm 0.02^{\text{a},\text{A}}$
MC	0	$3879 \pm 72^{\text{b},\text{A}}$	$16.25 \pm 0.40^{\text{b},\text{A}}$	$41.91 \pm 1.43^{\text{a},\text{B}}$	$0.89 \pm 0.01$	$1.91 \pm 0.01^{\text{b},\text{A}}$
	2	$4728 \pm 290^{\text{bc},\text{B}}$	$10.72 \pm 0.28^{\text{a},\text{B}}$	$22.72 \pm 1.33^{\text{a},\text{A}}$	$0.90 \pm 0.01$	$1.92 \pm 0.00^{\text{a},\text{A}}$
HAR	0	$2892 \pm 418^{\text{a},\text{A}}$	$18.07 \pm 0.63^{\text{bc},\text{A}}$	$63.51 \pm 10.52^{\text{b},\text{B}}$	$0.90 \pm 0.01$	$1.94 \pm 0.00^{\text{b},\text{A}}$
	2	$3670 \pm 287^{\text{a},\text{A}}$	$16.84 \pm 0.50^{\text{b},\text{A}}$	$46.14 \pm 4.72^{\text{c},\text{A}}$	$0.88 \pm 0.01$	$1.93 \pm 0.01^{\text{a},\text{A}}$

Different lowercase letters represent significantly different values within a column within a NaCl level between each flour cultivar  $p < 0.05$ . Different uppercase letters represent significantly different values within a column within a single flour cultivar comparing NaCl levels  $p < 0.05$ . NOTE: for circularity there are no significant differences  $p > 0.05$ .

Total particle area is associated with the total area covered by the particles counted. For total particle area the main effects of flour cultivar ( $p < 0.001$ ) and NaCl level ( $p < 0.01$ ) were both significant along with their interaction ( $p < 0.01$ ). There appears to be no trend among the cultivars when comparing 0% NaCl to 2% NaCl. Pembina has an increase in total area whereas, Roblin, McKenzie and Harvest have a decrease in total area, however Harvest's decrease is not significant ( $p > 0.05$ ). With that said, Pembina and Roblin approach a similar total particle area with the inclusion of 2% NaCl. Increase in total particle area could be a result of decreased gluten protein hydration and greater protein-protein interaction for Pembina forming a stronger gluten network, which is apparent by rheological findings. Jekle and Becker (2011) reported a decrease in total particle area with the increase in water addition to doughs and they explained this by a dilution effect on the gluten proteins due to increased hydration and increased water uptake by the starch granules which would hinder the gluten network formation. This is a similar explanation for Pembina with an increasing total particle area with the addition of salt due to charge shielding on the proteins and thus greater gluten protein interaction due to a decrease in protein hydration and thus an increase in gluten network formation covering a greater area. However the significant decrease in McKenzie's total particle area with the inclusion of NaCl down to a value lower than that of Pembina with no salt is opposite of what would be expected given that salt creates less protein hydration through charge shielding, causing increased protein-protein interaction, which visually it appears to do. It is also interesting to note that at the 2% NaCl level Pembina, Roblin and Harvest do not have significantly different total particle areas, however visually their microstructures appear different with Pembina and Roblin having a unidirectional organized gluten network, whereas Harvest appears to have a more porous and multidirectional gluten network. Therefore, the total particle area may not be a good indicator of gluten network formation within the present study.

Average particle size is the average size of all the particles counted. For average particle size the main effects of flour cultivar and NaCl level are highly significant ( $p < 0.001$ ) along with their interaction ( $p < 0.05$ ). The overall trend for doughs prepared with the different flour cultivars when comparing 0% NaCl to 2% NaCl is that the average particle size decreases with the inclusion of salt for each flour cultivar except Pembina, which has an average particle size that remains similar. The decrease in particle size with the inclusion of salt is indicative of less

aggregation of the gluten proteins and the creation of more gluten protein polymer strands unraveling and interacting to form the gluten network due to charge shielding.

Circularity is a descriptive parameter of the shape of the particles counted. A value closer to 1 indicates a more circular particle with a value of 1 being completely circular, whereas a value closer to 0 indicates a more elongated shape. The main effects of flour cultivar and NaCl level were not significant and neither was their interaction ( $p>0.05$ ). All the values for doughs prepared with the four different cultivars at the 0% and 2% NaCl level had values around 0.9 which indicated particles that were close to perfect circles, thus this parameter does not appear to give a good distinction between gluten microstructure as it was expected that those particles that formed longer gluten polymers would give more elongated shapes with values closer to 0 and those gluten protein particles that were aggregated, forming a weak gluten network would have given values closure to a circular shape. Jekle and Becker (2011) reported circularity values ranging from ~0.77 to 0.83 for doughs with increasing water additions. They concluded that the parameter of circularity showed a high fluctuation and therefore a low significance.

According to image j analysis fractal dimension is a measure of the degree of an image's pattern or complexity. The main effect of flour cultivar was significant ( $p<0.01$ ), however NaCl level was not ( $p>0.05$ ) but their interaction was significant ( $p<0.05$ ). Within papers by Jekle and Becker (2011) and Bigne et al. (2016) fractal dimension (FD) relates to complexity of images and a higher FD indicates a higher complexity or a more developed and filamentous network. However within the present study, a higher FD value did not translate into a more complex or developed gluten network formation when compared to the visual appearance of the micrographs. When comparing images of 2% NaCl Pembina to 2% Harvest it was expected that Pembina would have the higher FD value given a more formed gluten network and Harvest would have a lower value given the high aggregation of gluten proteins and porous structure. The overall trend for doughs prepared at 0% NaCl with the different flour cultivars all had similar FD values (~1.93) ( $p>0.05$ ) except for Pembina which had the lowest FD (1.85) ( $p<0.05$ ). Dough prepared with 2% NaCl with the different cultivars were found to have significantly similar FD values (~1.91) ( $p>0.05$ ). From these results it is presumed that Harvest has the significantly higher FD value (1.93) than Pembina (1.87) when comparing the main effect of flour cultivar, regardless of NaCl level, because it fills the space more with the larger aggregates (as apparent in the micrograph with a very dense/ porous structure). Whereas, Pembina, has a smaller FD given

that it is more organized and has more empty space in between the protein polymer strands creating the network. The FD values relate to the total particle area with respect to space filling. If there is a higher total particle area then the FD would also be higher which helps explain why Harvest would have the higher value than Pembina when comparing flour cultivars, regardless of NaCl level, given that Harvest also has a significantly higher total particle area than Pembina.

#### **4.5 CONCLUSIONS**

Consistently throughout the analysis, the magnitude of the effect of NaCl level was determined by the effect of flour cultivar with regard to the dough rheological properties, dough stickiness, dough morphology and water mobility. Pembina had the lowest protein content, however consistently produced dough with the greatest dough strength/gluten network with the lowest dough stickiness in the reduced sodium formulations. Whereas the flour cultivar of Harvest, which has the second highest protein content, consistently produced dough with the weakest dough strength/gluten network with the greatest dough stickiness in the low sodium formulations. Therefore, it was concluded that quality of gluten plays a greater role in mitigating dough stickiness as opposed to gluten content. It was also seen that some flour cultivars were more sensitive to changes in sodium chloride levels with Harvest appearing to be more affected by the NaCl level changes and Pembina appearing to be the least sensitive.

With respect to water mobility although the freezable water content for each of the flour cultivars did not see major changes at the different NaCl levels it did point to the conclusion that there are changes in the free water's location within the dough when viewed in combination with the dough stickiness results and the TGA curve fitting results. Without the addition of NaCl to the formulation, the water's location is near the surface of the dough in the diffusive layer (free and weakly absorbed water) which would increase the adhesive forces between the dough surface and the mixing surface, resulting in an increase in dough stickiness. However, with the addition of NaCl the water's location changes from being associated near the surface/weakly absorbed and becomes more strongly entrapped multilayer water within the stronger formed gluten network. The greater gluten-gluten polymer interactions, as a result of NaCl, increase cohesive forces within the dough and the change in the water's location decreases the adhesive forces, decreasing the dough stickiness. Therefore when looking at the TGA results in combination with the DSC results it is concluded that although the water that is changing

location or association with the dough components it still remains as unbound water that is freezable, but it is the change from being near the surface and weakly absorbed to either being associated with the starch or gluten fraction as unbound water (more strongly absorbed multilayer water, less mobile), when NaCl is added, that impacts dough stickiness. Based on the TGA deconvolution findings it was concluded that the gluten network may not be the sole factor governing dough stickiness in low sodium environments and instead stickiness is governed by the relationship between free water and water associated with the starch-fraction. With that said the strength of gluten network may mask the changes in the water's association which is why the cultivar Pembina results in less stickiness in low sodium environments. This leads to the conclusion that increased screening and greater attention to flour blending may be necessary when facing sodium reduction in the bread industry.

#### **4.6 LINKAGE TO CHAPTER 5**

Findings from this study gave foundational knowledge of the importance in gluten protein quality when mitigating the effects of sodium reduction in dough formulations. The high relevance of flour cultivar in relation to NaCl reduction effects on dough strength and water mobility indicates the need for further examination to consider the impact of the nature of interactions occurring within the gluten network. As such, the impact of alternative salts to NaCl on dough rheology, stickiness and water mobility within the dough was investigated to shed greater insight into the gluten properties under a low sodium environment.

## **5. EFFECT OF SALTS FROM THE LYOTROPIC SERIES ON THE HANDLING PROPERTIES OF DOUGH PREPARED FROM PEMBINA AND HARVEST CWRs WHEAT CULTIVARS**

### **5.1 ABSTRACT**

The influence of select salts from the lyotropic series ( $\text{NH}_4\text{Cl}$ ,  $\text{KCl}$ ,  $\text{NaCl}$ ,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ , and  $\text{MgSO}_4$ ) on the rheology, stickiness, morphology and water mobility of dough prepared from a strong (Pembina) and weak (Harvest) dough producing CWRs flour were examined at a 1 and 2% level. Overall, Pembina was found to develop stronger gluten networks that were more resistant to imposed stress than Harvest as evident by a lower  $\tan \delta$  and reduced amount of deformation (creep compliance [ $J_{\max}$ ]) during creep recovery. However the effect of salt-type was different depending on the cultivar. In the case of Pembina,  $\text{MgCl}_2$  and  $\text{MgSO}_4$  resulted in lower complex moduli values relative to those with  $\text{NaCl}$ , whereas all other salt-types were similar to dough containing  $\text{NaCl}$ . In the case of Harvest, greater salt sensitivity was observed where the addition of  $\text{KCl}$ ,  $\text{CaCl}_2$  and  $\text{MgCl}_2$  resulted in a weaker gluten network (lower complex modulus [ $|G^*|$ ]) associated with increased hydration of the gluten. In the case of Pembina, only dough prepared with  $\text{NH}_4\text{Cl}$  was found to have significantly reduced  $J_{\max}$  relative to those with  $\text{NaCl}$ , whereas all other salt-types were similar. For Harvest,  $\text{KCl}$ ,  $\text{CaCl}_2$ , and  $\text{MgCl}_2$  all showed a weakening effect on the gluten network as indicated by higher  $J_{\max}$  values relative to  $\text{NaCl}$ . In contrast,  $\text{NH}_4\text{Cl}$  and  $\text{MgSO}_4$  resulted in lower  $J_{\max}$  values relative to  $\text{NaCl}$ . Overall Pembina showed lower dough stickiness than Harvest in all cases. For both Pembina and Harvest, dough stickiness was found to show the greatest decrease with the addition of  $\text{NH}_4\text{Cl}$ . Interestingly, in the case of water mobility the alternative salts seemed to have a cultivar dependent effect with respect to a change of free water to either the starch-fraction or the gluten-fraction. Enhanced dough morphology was noticed for Pembina and Harvest in the presence of  $\text{NH}_4\text{Cl}$ , whereas  $\text{MgCl}_2$  resulted in a detrimental effect for Pembina but a positive effect on Harvest which could be due to the effect of the alternative salts effect on water movement between the dough components.

## 5.2 INTRODUCTION

The need to decrease sodium chloride within food products due to adverse health effects of high sodium diets has left the food industry looking to alternatives to achieve the same functionality that sodium chloride imparts to food products. For bread, the biggest problem for industrial bread producers is the phenomenon of sticky dough with the reduction of NaCl in dough formulations. The presence of dough stickiness occurs when adhesive forces (i.e., between the dough surface and the mixing surface) are greater than the cohesive forces (i.e., protein-protein interactions within the dough) (Hoseney & Smewing, 1999; Adhikari et al., 2001; van Velzen et al., 2003). Sodium chloride works in bread dough to enhance flavour, lengthen shelf life, form a stronger gluten network, control yeast fermentation, therefore controlling the rate of gas production, and ultimately enhances the final bread loaf quality to produce high loaf volume with a uniform and fine crumb grain (Miller & Hoseney, 2008; Belz et al., 2012). A reduction in NaCl decreases the quality of the final bread product in terms of texture, volume, flavour and colour. The poor quality texture and volume results because of the negative impact salt reduction has on dough rheology and handling properties as a consequence of the dough sticking to mixing surfaces and other processing equipment (Farahnaky & Hill, 2007). Alternative salts from the lyotropic series (also known as the Hofmeister series) have been examined to replace either the cation (sodium) or anion (chloride) in hopes of producing the same effects as NaCl on dough handling properties and final loaf volume and quality (Salovaara, 1982ab; Kinsella & Hale, 1984; Preston, 1989; He et al., 1992; Butow et al., 2002; Charlton et al., 2007; Braschi et al., 2009; Kaur et al., 2011; Uthayakumaran, 2011; Tuhumury et al., 2016ab). If similar dough rheology in terms of gluten network strength and dough handling properties could be achieved this would mitigate the issue of dough stickiness with the reduction or removal of NaCl in dough formulations.

Examining the effects that salts from the lyotropic series have on the dough rheology and dough handling properties could lead to a greater understanding of the nature of interactions (i.e., hydrogen and hydrophobic bonding) occurring within the dough matrix under low sodium conditions (Salovaara, 1982a; Preston, 1989; Miller & Hoseney, 2008). The lyotropic series ranks ions based on their ability to cause protein aggregation (precipitation) or disassociation (solubility) within solution (Salovaara, 1982a; Preston, 1989; Miller & Hoseney, 2008; Tuhumury et al., 2016a). The anions and cations are ranked from most stabilizing to most

destabilizing, with respect to their effect on protein-protein and protein-water interactions, and are classified as non-chaotropic and chaotropic (He et al., 1992; Miller & Hosney, 2008). Non-chaotropic ions are stabilizing ions which cause a decrease in protein hydration, an increase in structure and a decrease in solubility (i.e., ion-water interactions are favoured thus protein-protein interactions are increased). Chaotropic ions are destabilizing ions which cause an increased hydration and an increase in protein solubility (i.e., protein-water interactions are favoured, thus protein-protein interactions are decreased). Therefore, the level of hydrophobic interactions and hydrogen bonding within the dough matrix can be altered depending on which anions or cations are present (Preston, 1989; Butow et al., 2002; Miller & Hosney, 2008). Given that the non-chaotropic ions promote protein-protein interactions and water structuring these cations may be useful in maintaining the gluten network and thus dough strength, therefore decreasing the occurrence of stickiness with the removal of NaCl.

However, it is the cation sodium that is linked to adverse health effects; therefore, the focus will be on examining the effect of the cation not the anion. Salovaara et al. (1982a), Kaur et al. (2011), Uthayakumaran (2011), and Tuhumury et al. (2016a) all examined the effect of replacement of sodium with other cations from the lyotropic series on the dough strength and rheology. All seemed to find that the effect of the cations followed the ranking of the lyotropic series  $\text{NH}_4^+ > \text{K}^+ = \text{Na}^+ > \text{Ca}^{2+} > \text{Mg}^{2+}$ . They all also found that KCl had the most similar effect to NaCl, however in these studies dough stickiness was not analyzed and there was also the drawback of the metallic flavour reported with KCl. The aim of this research was to examine the effect of salts from the lyotropic series, compared to NaCl, on the prevention of dough stickiness utilizing a flour cultivar known to form non-sticky doughs at decreased sodium chloride levels compared to a flour cultivar known to form sticky doughs at reduced sodium chloride levels. The impact of salt replacement on the dough rheology, morphology and water mobility was also to be examined to give a link between stickiness and the gluten network formation and water movement within the dough.



## 5.3 MATERIALS AND METHODS

### 5.3.1 Materials

Pembina and Harvest CWRS wheat cultivars (were kindly provided the Crop Development Centre at the University of Saskatchewan (Saskatoon, SK). The two cultivars were grown in plots at the University of Saskatchewan's Kemen Crop Research Farm under rain-fed conditions on fallowed land. The previous crops in the rotation were canola, pea, and barley. Cultivars were seeded May 21, 2013 and each cultivar was grown in a 2.4 m wide by 15.2 m long plot with row spacing of 17.8 cm. The herbicide, fungicide and fertilizer used were: BuctrilM (1.0 L/ha) [applied June 11, 2013], Headline (0.4 L/ha) [applied June 28, 2013], and 56.0 kg/ha 23-23-0-10 [applied with seed], respectively. For seed conditioning: 13/64 round holed screen to scalp, 5.5 X ¾ slotted and 12 triangle buckwheat screens to sift were used. The cleaned amounts for each cultivar were: Pembina-40 kg and Harvest-60 kg. Pembina was developed at the Canada Department of Agriculture Research Station (Winnipeg, MB, Canada) by the Rust Area Project Group; it is unlikely to be grown outside of the rust area and is best suited to the Red River Valley (Campbell, 1963). Pembina is a cultivar that has an excellent baking quality, a yield comparable to that of Selkirk grown in the Red River Valley, slightly more rust resistance than Selkirk and has a one day earlier maturation than Selkirk (Campbell, 1963). Harvest is a cultivar that was developed by Agriculture and Agri-Food Canada (Cereal Research Centre, Winnipeg, MB, Canada) that is suitable to the wheat-producing regions of the Prairies (Fox et al., 2010). Harvest has the qualities of high-yield, high test weight and preharvest sprouting resistance (Fox et al., 2010). Milling of the four selected wheat cultivars was done on a Buhler MLU202 Experimental Mill according to the American Association of Cereal Chemists International approved method 26-21.02. Prior to milling the grain was tempered to a moisture of 15.5% for ~ 16 hrs.

Deionized and distilled water (DDH<sub>2</sub>O) was used in all dough formulations. Salts (NH<sub>4</sub>Cl, KCl, NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub>) and paraffin oil were of ACS reagent grade and were purchased from VWR (Edmonton, AB, Canada). Rhodamine B was purchased from Sigma Aldrich (Oakville, ON, Canada). The water used in this research was produced from a Millipore Milli-Q™ water purification system (Millipore Corp., Milford, MA, USA).

### 5.3.2 Rheological analysis

#### 5.3.2.1 Dough preparation

All dough was prepared using a 10 g mixograph (TMCO National Mfg., Lincoln, NE) utilizing a dough formulation comprised of flour (weight on a 14% moisture basis), water (weight based on farinograph absorption results) and salts from the lyotropic series (NH<sub>4</sub>Cl, KCl, NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub>) at 1 and 2% level (on flour weight basis). All doughs were mixed until just after reaching peak time. All doughs were prepared in triplicate.

#### 5.3.2.2 Dough rheology

Dough rheology testing was performed according to Jekle and Becker (2011) using an AR-1000 rheometer (TA Instruments, New Castle, USA) equipped with a 40 mm parallel plate fixture (2 mm gap). The temperature was kept constant at 30°C using a Peltier plate temperature system. Dough (~5 g) was weighed out and then placed in-between the two plates. The excess dough that resulted once the parallel plate fixture was lowered was trimmed away using a plastic spatula and paraffin oil was applied to the free surface of the dough to prevent the dough from drying out. A resting period of 10 min was utilized for the dough prior to any measurements taken. The first test that was run was the oscillatory frequency sweep followed by the creep recovery test.

*Oscillatory frequency sweep:* The frequency was varied from 0.10 to 100.00 Hz at a constant amplitude strain of 0.03% which was determined to be within the linear viscoelastic regime based on preliminary testing. The latter was determined using a strain sweep to determine at which strain the material deviates from linearity. The dynamic storage ( $G'$ ) and loss ( $G''$ ) moduli of the dough samples were measured. Values were reported for 1 Hz. From these measurements the loss factor was calculated using the following equation:

$$\tan \delta = G''(G')^{-1} \quad (\text{Eq. 5.1})$$

*Creep recovery test:* In the creep phase a constant shear stress  $\tau_0$  of 250 Pa at 30°C was applied to the dough sample for the duration of 180 s and then removed ( $\tau_0 = 0$  Pa). The

recovery/relaxation phase of the dough it was recorded for 360 s. The strain values were collected as a function of time and the final data was given in terms of compliance:

$$J(t) = \gamma(t) \tau_0^{-1} \quad (\text{Eq. 5.2})$$

where  $J$  is the compliance,  $\gamma$  strain, and  $\tau_0$  the constant stress which was utilized during the creep phase. The parameters of the creep phase measurements include the time and stress dependent recoverable shear deformation, the creep compliance  $J_{\max}$  (at  $t=180$  s of the creep phase). The creep recovery compliance  $J_r$  (at  $t=360$  s of the recovery phase) is a measure of the material's elasticity. The recovery compliance describes the mechanical energy that is stored in the sample during the creep phase. The relative elastic part  $J_{el}$  [-] was calculated by utilizing:

$$J_{el} = J_r (J_{\max})^{-1} \quad (\text{Eq. 5.3})$$

### 5.3.3 Dough Stickiness

Dough stickiness was measured according to the Chen and Hoskeney method (1995a) using a stable microsystems (SMS) TA.XTplus Texture profile analyzer equipped with a 5 kg load cell, using a Perspex cylinder 25.0 mm probe adhesion fixture and the SMS/Chen-Hoskeney dough stickiness cell. The dough samples were loaded into the cell and then extruded through the openings of the mesh screen by turning the dial on the bottom of the cell. A plastic spatula was then utilized to clean the initial extruded dough from the screen surface. To keep the dough height as consistent as possible, the dial was turned two thirds of the way, giving ~ 1 mm of dough height. After extrusion a plastic cover was placed over top of the dough, to reduce moisture loss, as it rested for 30 s. The cover was then removed and the probe was brought down to about 1 mm above the dough surface and then the test was run using a pre-test speed of 0.5 mm/s, test speed of 0.5 mm/s, post speed 10.0 mm/s, distance 15.0 mm, force 40.0 g, time 0.1 s, and trigger force 10.0 g. The force required by the Perspex probe to separate from the dough's surface was recorded as the dough stickiness measurement. Doughs were run in triplicate with each trial containing approximately six runs from one loading of the cell.

#### **5.3.4 Differential scanning calorimetry (DSC)**

Freezable water content was determined according to Lu and Seetharaman (2013) utilizing a Q series DSC Q2000 (TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling system and nitrogen as the purge gas. Dough samples were prepared using Pembina and Harvest cultivars with a 1% salt level using salts from the lyotropic series ( $\text{NH}_4\text{Cl}$ ,  $\text{KCl}$ ,  $\text{NaCl}$ ,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$  and  $\text{MgSO}_4$ ). Briefly, dough samples of ~ 10-20 mg were weighed into aluminum DSC pans which were then hermetically sealed before analysis and an empty pan was used as the reference. The reference pan and sample pan were then loaded and equilibrated at 30°C for 5 min. The pans were cooled from 30°C to -40°C at a rate of 10°C/min, held at -40°C for 5 min, followed by heating from -40°C to 40°C at a rate of 10°C/min. The melting peak enthalpy ( $\Delta H$ ) was obtained using the Universal Analysis 2000 version 4.5A software (TA instruments, New Castle, DE, USA). The freezable water content was calculated from the melting enthalpy peak divided by the enthalpy of pure water (333.55 J/g) and reported on a dry weight basis. Moisture determination was done on each dough in triplicate using 1% range of salts from the lyotropic series and Pembina and Harvest cultivars only (representing a strong and weak gluten network, respectively).

#### **5.3.5 Thermogravimetric analysis (TGA)**

Thermogravimetric analysis was performed on dough using TGA Q500 apparatus (TA Instruments, New Castle, DE, USA). Dough samples were prepared using Pembina and Harvest flours with 1%  $\text{NH}_4\text{Cl}$ ,  $\text{NaCl}$ , and  $\text{MgCl}_2$  on weight/weight basis. Dough samples (~70 mg) were placed on platinum pans and scanned from 25 to 200 °C at a rate of 5 °C/min. The weight change (which was attributed to moisture loss) and derivative of weight loss as a function of temperature were obtained. The derivative thermogravimetric (DTG) curve was used to differentiate between diffusive water (weakly absorbed free water entrapped near the surface of the dough; more mobile) and more tightly entrapped (more strongly absorbed multilayer water; less mobile) or bound water within the dough as done by Fessas and Schiraldi (2001), Crockett et al. (2011) and Roozendaal et al. (20012). The DTG traces were deconvoluted into Gaussian distributions using PeakFit software (Systat Software, Inc., San Jose, CA, USA) based on the method of Roozendaal et al. (2012) to give an indication of water's association with dough components of starch and gluten.

### 5.3.6 Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy was utilized to visualize the gluten network formation of dough samples prepared using Pembina and Harvest flours with 1% and 2%  $\text{NH}_4\text{Cl}$ ,  $\text{NaCl}$ , and  $\text{MgCl}_2$  on weight/weight basis and was based off of the method by Jekle and Becker (2011). The doughs were prepared as described above. The fluorescent dye rhodamine B was prepared at a concentration of 0.001% (w/v) in  $\text{DDH}_2\text{O}$  and was then utilized as the stock water addition to each dough formulation to ensure that the dye was distributed homogeneously throughout the dough during the mixing process. A small sample was then taken from the dough with two plastic spatulas to try to prevent stretching of the dough, the sample was about 4-5 mm in diameter. The dough sample was then loaded on the slide and flattened with the cover slip by placing the cover slip overtop and then turning the slide over and lightly pushing it down on the table. All samples were loaded this way. The dough was analyzed by a Nikon C2 CLSM microscope (Nikon, Mississauga, ON, Canada) using 20x Plan Fluor (numerical aperture 0.75, Nikon) objective lens. The excitation and emission wavelengths were 543 nm and 625 nm (rhodamine B), respectively. A total of 10 images with  $512 \times 512 \mu\text{m}$  pixel resolution were taken for each dough sample from different positions on the  $xy$ -axis. Each dough sample was analyzed in duplicate.

### 5.3.7 Statistical analysis

A three-way analysis of variance (ANOVA) was used to evaluate the effect of flour cultivar, salt-type and salt level for most of the parameters studied ( $|\text{G}^*|$ ,  $\tan \delta$ ,  $J_{\text{max}}$ ,  $J_{\text{el}}$  and stickiness). A two-way ANOVA was used to test the effect of flour cultivar and salt-type on the amount of freezable water content and TGA parameters. Since all parameters showed a significant two-way interaction between flour cultivar and salt-type, a subsequent one-way ANOVA using a Scheffe Post-hoc test was performed to test for differences. The statistical analysis program IBM SPSS (Armonk, NY, USA) was utilized.

## 5.4 RESULTS AND DISCUSSION

### 5.4.1 Dough rheology

#### 5.4.1.1 Oscillatory shear

Dough was prepared using Pembina and Harvest in the presence of various salts from the lyotropic series at the 1 and 2% (by flour weight) level, and examined using dynamic oscillatory shear rheology. Although the dynamic storage and loss moduli were measured, data was reported in the form of the  $|G^*|$  (complex modulus) (Figure 5.1) and  $\tan \delta$  parameters to give a combined analysis of the elastic and viscous components of the doughs. An analysis of variance of  $|G^*|$  (complex modulus) values found that the main effects of salt-type ( $p < 0.001$ ), salt level ( $p < 0.05$ ) and flour cultivar ( $p < 0.001$ ) were all significant, along with the two-way interaction of salt-type\*flour cultivar ( $p < 0.01$ ) (Table 5.1). All other interactions were not significant ( $p > 0.05$ ). Overall, doughs prepared with 2% salts (regardless of its type and flour) resulted in greater  $|G^*|$  (7182.50 Pa) relative to the 1% salt level ( $|G^*| = 6961.75$  Pa). Furthermore, overall doughs' prepared from the Pembina ( $|G^*| = 8867.03$  Pa) formed stronger gluten networks as indicated by the higher  $|G^*|$  values than for dough prepared from Harvest ( $|G^*| = 5277.22$  Pa), however the effects of salt-type was different depending on the flour used. For Pembina, the addition of  $MgCl_2$  ( $|G^*| = 8028.67$  Pa;  $p < 0.001$ ) and  $MgSO_4$  ( $|G^*| = 8357.67$  Pa;  $p < 0.01$ ) resulted in lower  $|G^*|$  values relative to those with NaCl ( $|G^*| = 9144.50$  Pa) which present a weakening effect on the gluten network, whereas all other salt-types were similar in magnitude as the dough containing NaCl. In the case of Harvest, greater salt sensitivity was observed where the addition of KCl ( $|G^*| = 5318.50$  Pa;  $p < 0.01$ ),  $CaCl_2$  ( $|G^*| = 4496.83$  Pa;  $p < 0.001$ ) and  $MgCl_2$  ( $|G^*| = 4321.17$  Pa;  $p < 0.001$ ) resulted in a decrease in the magnitude of  $|G^*|$  relative to dough prepared with NaCl ( $|G^*| = 6092.00$  Pa). All other salt-types were similar in magnitude as doughs with NaCl ( $p > 0.05$ ).

The loss tangent for doughs prepared as a function of salt-type, flour cultivar and salt levels are shown in Figure 5.2. An analysis of variance of  $\tan \delta$  values found that the main effects of salt-type ( $p < 0.001$ ), salt level ( $p < 0.001$ ) and flour cultivar ( $p < 0.001$ ) were all significant, along with the two-way and three-way interactions of salt-type\*flour cultivar ( $p < 0.001$ ) and salt-type\*salt level\*flour cultivar ( $p < 0.05$ ), respectively (Table 5.1). Overall, doughs prepared with Pembina were more rigid ( $\tan \delta = 0.360$ ) than when prepared using Harvest ( $\tan \delta = 0.392$ ), however effects were different depending on the salt-type and salt level.

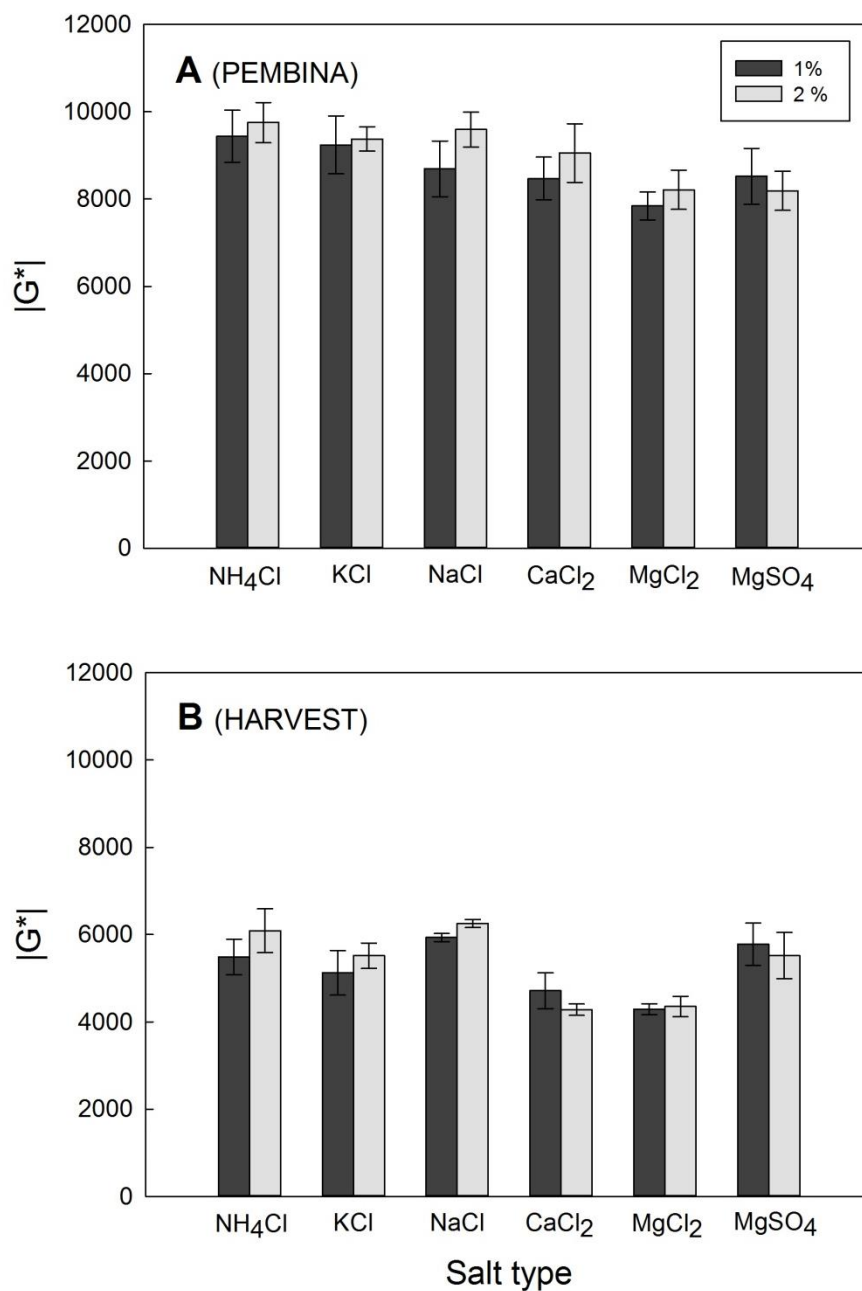


Figure 5.1 Complex modulus ( $|G^*|$ ) at 1 Hz for dough prepared using flours from Pembina (A) and Harvest (B) in the presence various salts from the lyotropic series (NH<sub>4</sub>Cl, KCl, NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub>) at the 1% and 2% levels (based on wt. flour). Data represent the mean  $\pm$  one standard deviation (n = 3).

Table 5.1 Probability values arising from a three-way analysis of variance for oscillatory, creep rheology, and stickiness data.

	$ G^* $	$\tan \delta$	$J_{\max}$	$J_{el}$	Stickiness
Main effects					
Salt-type	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Salt level	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Flour cultivar	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Interactions					
Salt-type*salt level	NS	NS	NS	NS	$p < 0.001$
Salt level*flour cultivar	NS	NS	NS	NS	$p < 0.05$
Salt-type*flour cultivar	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$
Salt-type*salt level*flour cultivar	NS	$p < 0.05$	NS	$p < 0.05$	$p < 0.001$



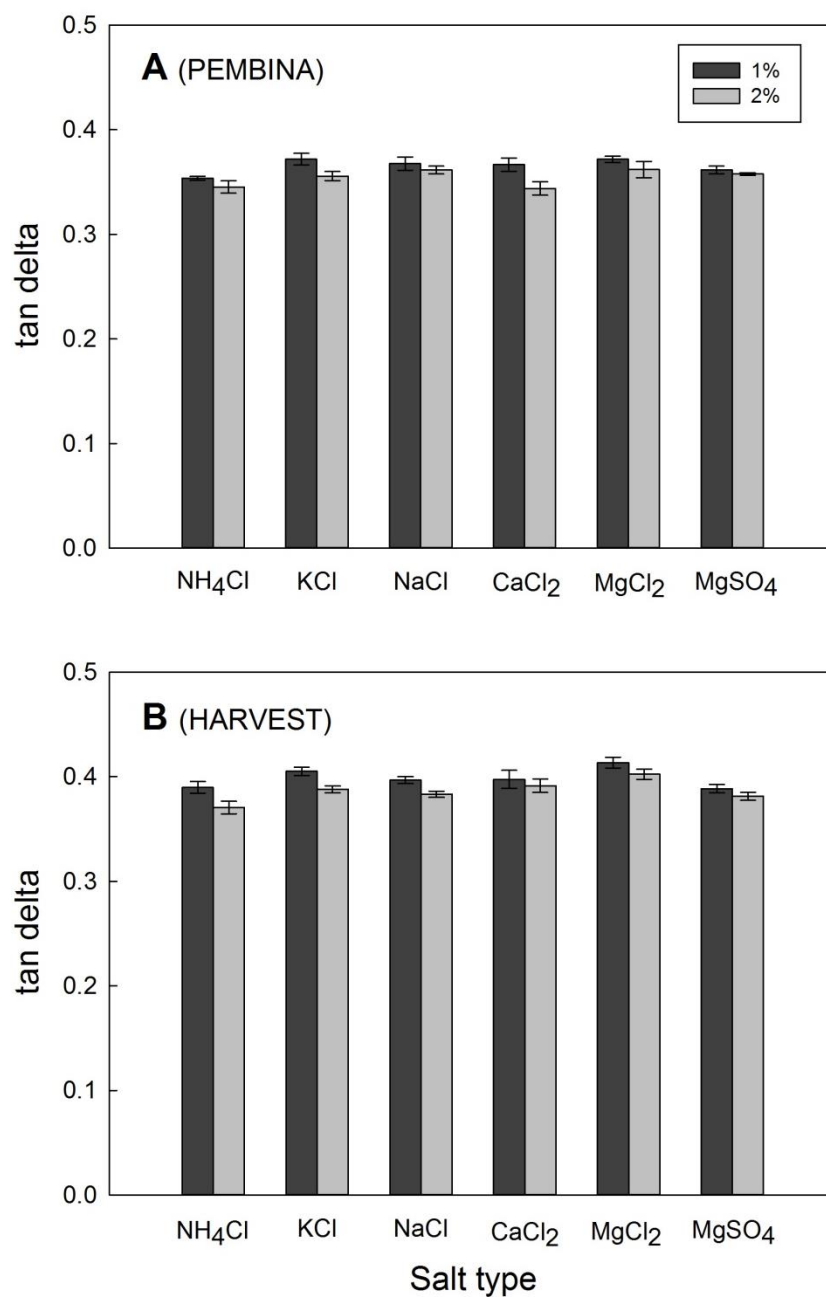


Figure 5.2 The loss tangent ( $\tan \delta$ ) at 1 Hz for dough prepared using flours from Pembina (A) and Harvest (B) in the presence various salts from the lyotropic series (NH<sub>4</sub>Cl, KCl, NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub>) at the 1% and 2% levels (based on wt. flour). Data represent the mean  $\pm$  one standard deviation ( $n = 3$ ).

In the case of doughs prepared by the Pembina, higher  $\tan \delta$  values were found at the 1% levels for KCl ( $p < 0.001$ ),  $\text{CaCl}_2$  ( $p < 0.001$ ), and  $\text{MgCl}_2$  ( $p < 0.05$ ) relative to the 2% salt levels suggesting that the doughs were becoming more viscous with a decrease in salt level, whereas  $\tan \delta$  values were similar in magnitude for doughs prepared with  $\text{NH}_4\text{Cl}$ , NaCl and  $\text{MgSO}_4$  regardless of the salt level added ( $p > 0.05$ ) (Figure 5.2A). When comparing different salt-types to NaCl, at the 1% level, only doughs prepared with  $\text{NH}_4\text{Cl}$  ( $p < 0.001$ ) gave lower  $\tan \delta$  values indicating increased protein-protein interactions and gluten strength. Whereas at the 2% salt level, doughs prepared with  $\text{CaCl}_2$  ( $p < 0.001$ ) and  $\text{NH}_4\text{Cl}$  ( $p < 0.001$ ) were the only ones to give lower  $\tan \delta$  values than NaCl.

In contrast, doughs prepared by the Harvest, higher  $\tan \delta$  values were found at the 1% levels for NaCl ( $p < 0.01$ ), KCl ( $p < 0.001$ ),  $\text{MgCl}_2$  ( $p < 0.05$ ) and  $\text{NH}_4\text{Cl}$  ( $p < 0.001$ ) relative to the 2% salt levels suggesting that the dough were becoming more viscous and weaker in nature at the lower salt levels (Figure 5.2B). Relative to the Pembina, dough prepared with the Harvest are much more sensitive to salt levels. When comparing different salt-types to NaCl at the 1% level, only doughs prepared with KCl ( $p < 0.05$ ) and  $\text{MgCl}_2$  ( $p < 0.001$ ) gave higher  $\tan \delta$  values indicating a weakening of the gluten network, whereas all others were similar in magnitude. At the 2% salt level, dough prepared with  $\text{NH}_4\text{Cl}$  ( $p < 0.01$ ) was the only salt-type to give lower  $\tan \delta$  values than NaCl, whereas  $\text{MgCl}_2$  gave higher ( $p < 0.001$ ).

#### 5.4.1.2 Creep recovery

Maximum deformation ( $J_{\max}$ ) of the dough during a creep-recovery experiment as a function of salt-type, salt level and flour cultivar is given in Figure 5.3. An analysis of variance of  $J_{\max}$  values found that the main effects of salt-type ( $p < 0.001$ ), salt level ( $p < 0.001$ ) and flour cultivar ( $p < 0.001$ ) were all significant, along with the two-way interaction between salt-type\*flour cultivar ( $p < 0.001$ ) (Table 5.1). Overall, doughs prepared with 2% salts (regardless of its type and flour) resulted in lower  $J_{\max}$  values ( $3.23 \text{ mPa}^{-1}$ ) relative to the 1% salt level ( $J_{\max} = 4.31 \text{ mPa}^{-1}$ ). The greater amount of NaCl would result in greater charge shielding and increased protein-protein interactions which would enable for a stronger formed network capable of resisting the deformation imposed. Furthermore, overall doughs' prepared from Pembina ( $J_{\max} = 1.89 \text{ mPa}^{-1}$ ) formed stronger gluten networks as indicated by the lower  $J_{\max}$  values than for dough

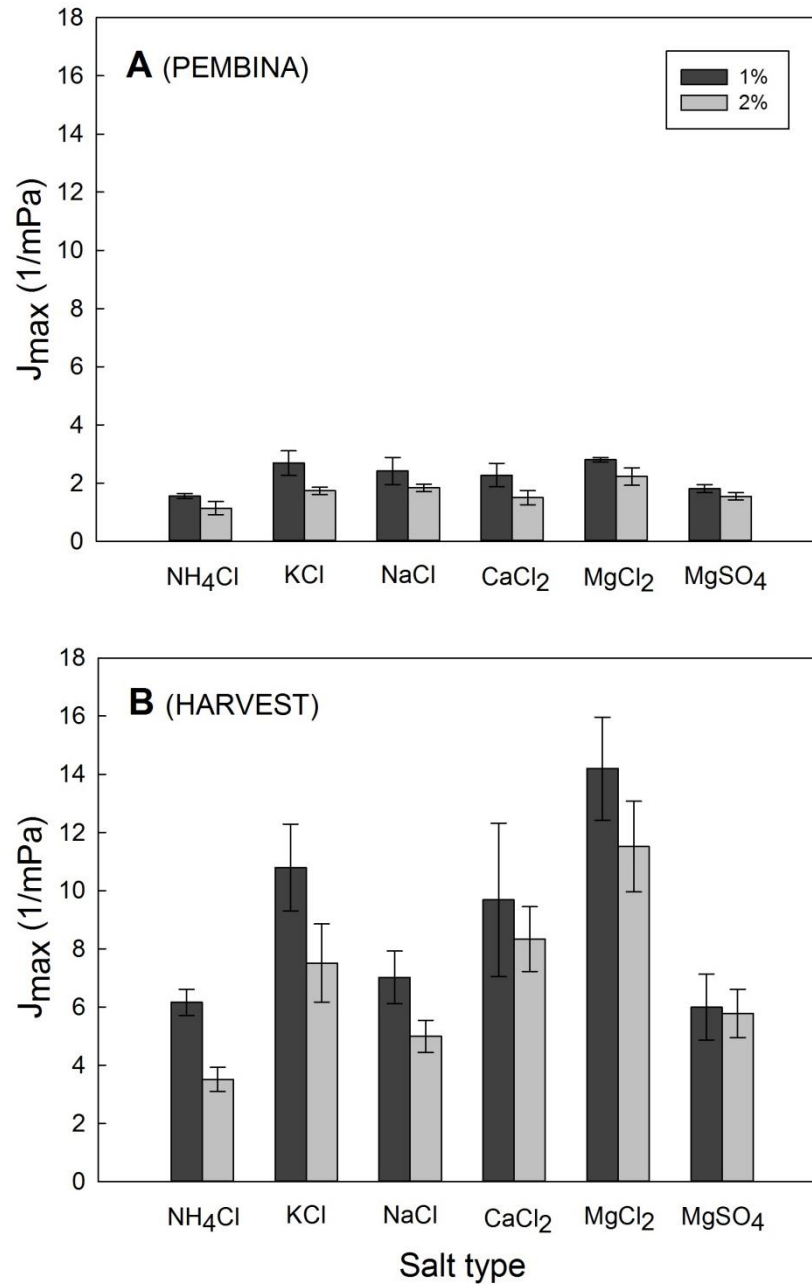


Figure 5.3 The creep compliance ( $J_{\max}$ ) for dough prepared using flours from Pembina (A) and Harvest (B) in the presence various salts from the lyotropic series (NH<sub>4</sub>Cl, KCl, NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub>) at the 1% and 2% levels (based on wt. flour). Data represent the mean  $\pm$  one standard deviation ( $n = 3$ ).

prepared from Harvest ( $J_{\max} = 7.36 \text{ mPa}^{-1}$ ), however the effects of salt-type was different depending on the flour used. In the case of Pembina, only doughs prepared with  $\text{NH}_4\text{Cl}$  were found to have significantly reduced  $J_{\max}$  values ( $p < 0.01$ ) relative to those with  $\text{NaCl}$ , whereas all other salt-types were similar in magnitude ( $p > 0.05$ ). For Harvest,  $\text{KCl}$  ( $p < 0.01$ ),  $\text{CaCl}_2$  ( $p < 0.01$ ), and  $\text{MgCl}_2$  ( $p < 0.001$ ) all showed a weakening effect on the gluten network as indicated by higher  $J_{\max}$  values relative to  $\text{NaCl}$ . In contrast,  $\text{NH}_4\text{Cl}$  and  $\text{MgSO}_4$  resulted in a similar  $J_{\max}$  values relative to  $\text{NaCl}$  indicating a similar strengthening effect.

The relative elasticity ( $J_{\text{el}}$ ) for doughs prepared as a function of salt-type, flour cultivar and salt levels is shown in Figure 5.4. An analysis of variance of  $J_{\text{el}}$  values found that the main effects of salt-type ( $p < 0.001$ ), salt level ( $p < 0.001$ ) and flour cultivar ( $p < 0.001$ ) were all significant, along with the two-way and three-way interactions of salt-type\*flour cultivar ( $p < 0.01$ ) and salt-type\*salt level\*flour cultivar ( $p < 0.05$ ), respectively (Table 5.1). Overall, doughs prepared with Pembina were more rigid ( $J_{\text{el}} = 0.594$ ) showing greater creep recovery than those prepared using Harvest ( $J_{\text{el}} = 0.311$ ), however effects were different depending on the salt-type and salt level.

In the case of doughs prepared with Pembina, higher  $J_{\text{el}}$  values were found at the 2% levels for  $\text{KCl}$  ( $p < 0.001$ ),  $\text{CaCl}_2$  ( $p < 0.01$ ),  $\text{MgCl}_2$  ( $p < 0.05$ ),  $\text{MgSO}_4$  ( $p < 0.01$ ) and  $\text{NH}_4\text{Cl}$  ( $p < 0.05$ ) relative to the 1% salt levels suggesting that the dough were more elastic and showed greater recovery, whereas  $J_{\text{el}}$  values were similar in magnitude for doughs prepared with  $\text{NaCl}$  regardless of the salt level added ( $p > 0.05$ ) (Figure 5.4A). This displays the effect of greater salt concentration on charge shielding to induce greater elasticity through increased protein-protein polymer interactions. When comparing different salt-types to  $\text{NaCl}$ , at the 1% level, only doughs prepared with  $\text{NH}_4\text{Cl}$  ( $p < 0.05$ ) gave higher  $J_{\text{el}}$  values indicating increased network elasticity. In contrast,  $\text{KCl}$  ( $p < 0.05$ ) and  $\text{MgCl}_2$  ( $p < 0.05$ ) showed reduced  $J_{\text{el}}$  values relative to  $\text{NaCl}$  suggesting that those salts caused a decrease in network recovery. At the 2% salt level, doughs prepared with  $\text{MgSO}_4$  ( $p < 0.01$ ) and  $\text{NH}_4\text{Cl}$  ( $p < 0.05$ ) were the only salts to give greater  $J_{\text{el}}$  values than  $\text{NaCl}$ , indicating an increased recovery of the network, whereas all other salt-types were similar in magnitude ( $p > 0.05$ ). This result displays the non-chaotropic effect of the  $\text{SO}_4^{2-}$  anion and the  $\text{NH}_4^+$  cation.

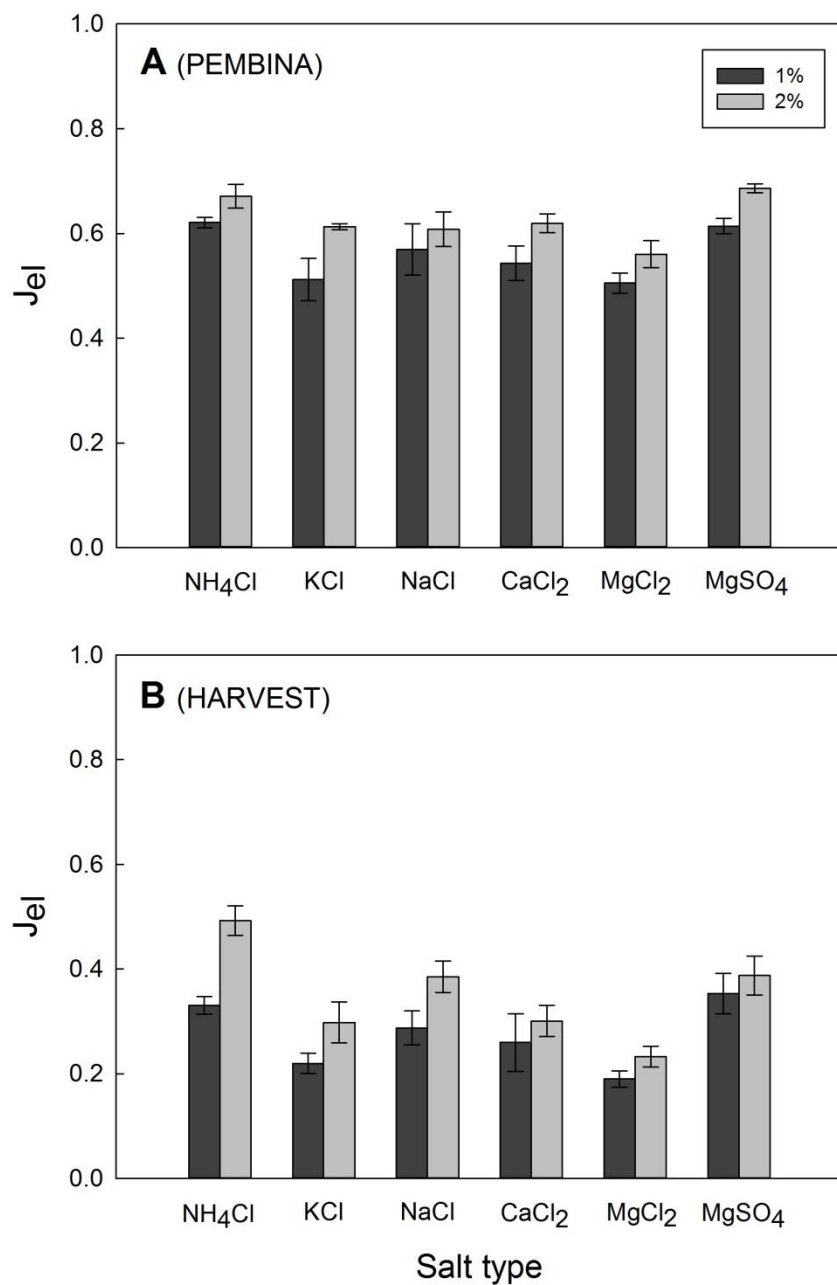


Figure 5.4 The relative elasticity ( $J_{el}$ ) for dough prepared using flours from Pembina (A) and Harvest (B) in the presence of various salts from the lyotropic series (NH<sub>4</sub>Cl, KCl, NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub>) at the 1% and 2% levels (based on flour weight). Data represent the mean  $\pm$  one standard deviation ( $n = 3$ ).

For doughs prepared by the Harvest, higher  $J_{el}$  values were found at the 2% levels for NaCl ( $p<0.001$ ), KCl ( $p<0.01$ ), and  $NH_4Cl$  ( $p<0.001$ ) relative to the 1% salt levels suggesting that doughs were becoming more viscous and weaker in nature at the lower salt levels (Figure 5.4B). All other salt-types were not influenced by the salt levels ( $p>0.05$ ). Relative to Pembina, doughs prepared with Harvest are much more sensitive to salt levels. When comparing different salt-types to NaCl, at the 1% level, only doughs prepared with KCl ( $p<0.01$ ) and  $MgCl_2$  ( $p<0.001$ ) gave lower  $J_{el}$  values indicating a weakening of the gluten network, whereas all others were similar in magnitude with the exception of  $MgSO_4$  ( $p<0.01$ ) which showed higher  $J_{el}$  values than NaCl. At the 2% salt level, dough prepared with  $MgSO_4$  ( $p>0.05$ ) had a similar effect on  $J_{el}$  compared to NaCl whereas those with KCl ( $p<0.01$ ),  $CaCl_2$  ( $p<0.01$ ) and  $MgCl_2$  ( $p<0.001$ ) led to dough with reduced  $J_{el}$  values relative to NaCl. Doughs prepared with  $NH_4Cl$  at the 2% levels were the only ones to experience an increase in  $J_{el}$  values ( $p<0.001$ ) when compared to NaCl, indicating an increasing dough elasticity and strength, displaying the non-chaotropic effect to induce greater protein-protein interactions through hydrophobic and hydrogen bonding.

#### 5.4.2 Dough stickiness

The stickiness of doughs prepared as a function of salt-type, flour cultivar and salt level are shown in Figure 5.5. An analysis of variance of stickiness values found that the main effects of salt-type ( $p<0.001$ ), salt level ( $p<0.001$ ) and flour cultivar ( $p<0.001$ ) were all significant, along with the two-way and three-way interactions of salt-type\*flour cultivar ( $p<0.001$ ), salt level\*salt-type ( $p<0.001$ ), flour cultivar\*salt level ( $p<0.05$ ) and salt-type\*salt level\*flour cultivar ( $p<0.001$ ), respectively (Table 5.1). Overall, doughs prepared with Pembina created doughs with less stickiness (0.364 N) than when prepared using Harvest (0.532 N), however effects were different depending on the salt-type and salt level. This is a continuing trend throughout the current research, with the rheological and stickiness results of Pembina, with higher gluten quality, exhibiting better dough handling properties than Harvest. Overall, when comparing salt level (regardless of flour cultivar and salt-type) doughs prepared with 1% salt (0.465 N) compared to the 2% level (0.431 N) noticed an increase in stickiness with the decrease in the amount of salt ( $p<0.001$ ) indicating a formation of a weaker gluten network at the lower salt level. When comparing salt-type to NaCl, regardless of salt level and flour cultivar, all

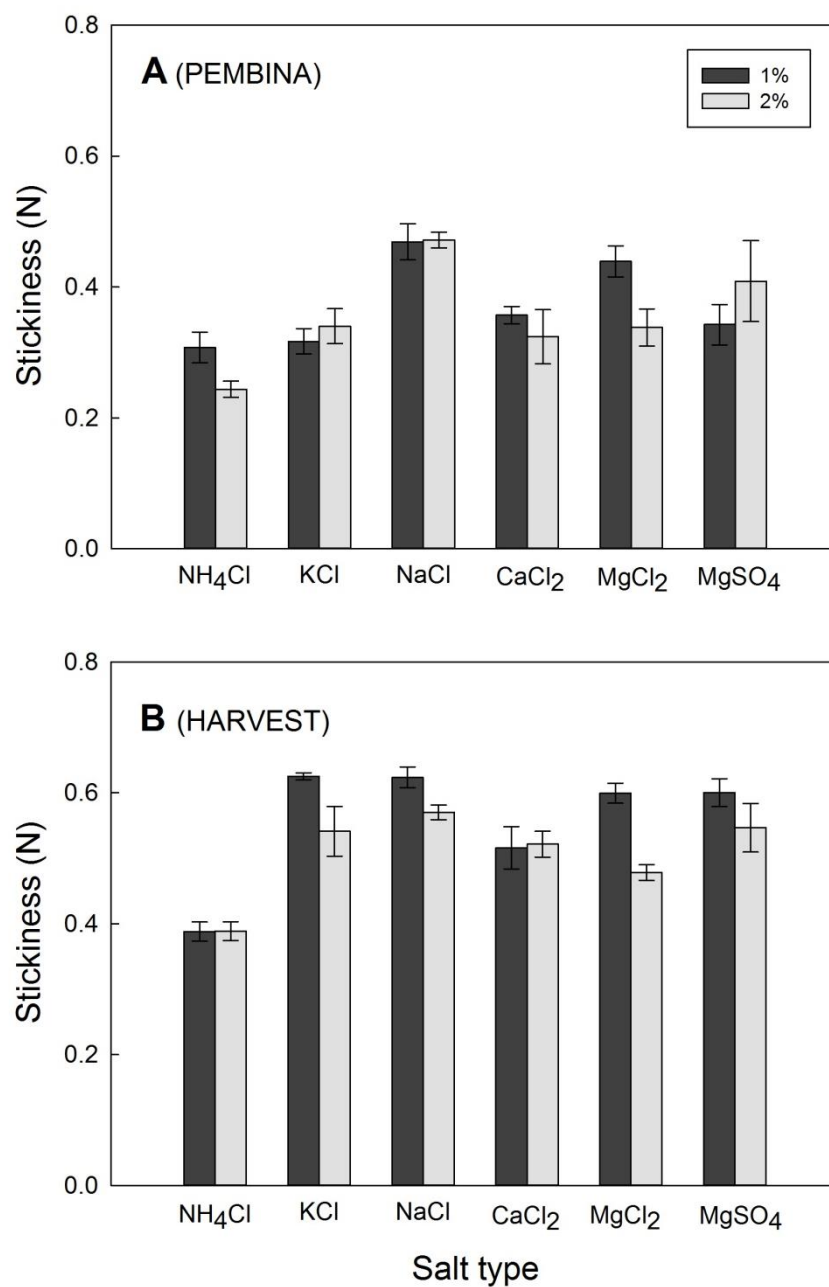


Figure 5.5 Stickiness values for dough prepared using flours from Pembina (A) and Harvest (B) in the presence of various salts from the lyotropic series (NH<sub>4</sub>Cl, KCl, NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub>) at the 1% and 2% levels (based on wt. flour). Data represent the mean  $\pm$  one standard deviation (n = 3).

alternative salts decreased dough stickiness compared to NaCl (0.533 N), with  $\text{NH}_4\text{Cl}$  (0.332 N) decreasing stickiness the most ( $p < 0.001$ ).

For doughs prepared with Pembina, higher stickiness values were found at the 1% levels for  $\text{NH}_4\text{Cl}$  ( $p < 0.01$ ) and  $\text{MgCl}_2$  ( $p < 0.001$ ) when compared to the 2% level, whereas decreased stickiness was found for  $\text{MgSO}_4$  ( $p < 0.01$ ) and no difference was noted for NaCl, KCl, and  $\text{CaCl}_2$  between the levels ( $p > 0.05$ ). When comparing different salt-types to NaCl at the 1% level only  $\text{MgCl}_2$  was not significantly different from NaCl ( $p > 0.05$ ) and all other salt-types significantly decreased dough stickiness ( $p < 0.001$ ). Whereas at the 2% level all alternative salts significantly decreased dough stickiness when compared to NaCl ( $p < 0.01$ ).

In contrast, for doughs prepared by Harvest, higher dough stickiness was found at the 1% levels for NaCl ( $p < 0.05$ ), KCl ( $p < 0.001$ ),  $\text{MgCl}_2$  ( $p < 0.001$ ), and  $\text{MgSO}_4$  ( $p < 0.05$ ) relative to the 2% salt levels whereas  $\text{CaCl}_2$  and  $\text{NH}_4\text{Cl}$  did not significantly differ between 1 and 2% levels ( $p > 0.05$ ). This shows that stickiness for Harvest is more sensitive to the decrease in salt level compared to Pembina, which is presumed to be due to the differences in flour composition and quality with Harvest having lower quality gluten which requires greater salt to induce increased dough handling. When comparing different salt-types to NaCl at the 1% level, only  $\text{CaCl}_2$  ( $p < 0.001$ ) and  $\text{NH}_4\text{Cl}$  ( $p < 0.001$ ) significantly decreased dough stickiness, whereas all other salt-types had similar stickiness to NaCl prepared doughs at the 1% level. For doughs prepared at the 2% level, only  $\text{MgCl}_2$  ( $p < 0.001$ ) and  $\text{NH}_4\text{Cl}$  ( $p < 0.001$ ) significantly decreased dough stickiness when compared to NaCl.

#### **5.4.3 Freezable water content (FWC)**

The amount of freezable water content (FWC) within the dough as a function of flour cultivar and salt-type was examined by differential scanning calorimetry at the 1% salt level, and given in Figure 5.6. An analysis of variance of the amount of FWC found the main effects of salt-type ( $p < 0.001$ ) and flour cultivar ( $p < 0.001$ ), along with their interaction ( $p < 0.01$ ) to be significant. Overall, doughs prepared with Pembina (FWC = 0.426 g ice/g d.b) were found to have lower FWC than those prepared with Harvest (FWC = 0.472 g ice/g d.b). It is noteworthy to report that Pembina and Harvest doughs were prepared with their optimal FABs and therefore had different water additions (61.9 % and 65.5% respectively). Overall, when comparing all salt-types to NaCl (regardless of flour cultivar) it was found that all salt-types (FWC =  $\text{NH}_4\text{Cl}$ , 0.440



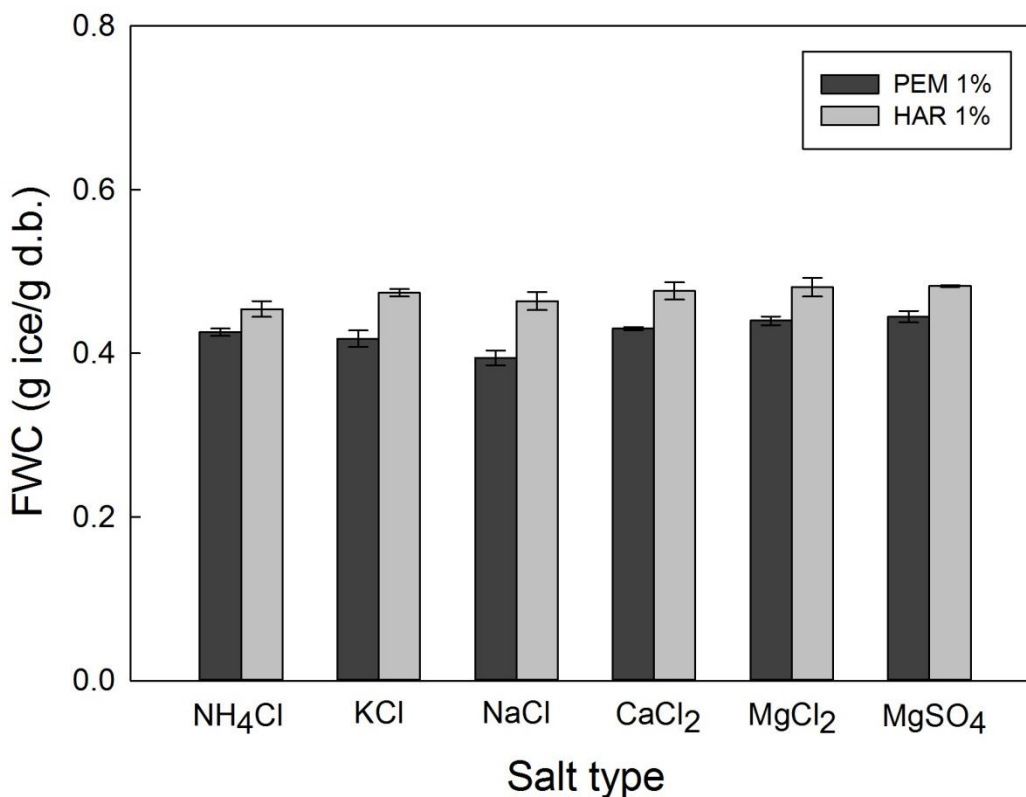


Figure 5.6 Freezable water content (FWC) as determined by differential scanning calorimetry for dough prepared using flours from Pembina (PEM) and Harvest (HAR) in the presence of various salts from the lyotropic series (NH<sub>4</sub>Cl, KCl, NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and MgSO<sub>4</sub>) at the 1% level (based on wt. flour). Data represent the mean  $\pm$  one standard deviation (n = 3).

g ice/ g d.b; KCl 0.446 g ice/ g d.b; MgCl<sub>2</sub> 0.460 g ice/ g d.b; CaCl<sub>2</sub> 0.453 g ice/ g d.b; and MgSO<sub>4</sub> 0.463 g ice/ g d.b) resulted in a significant increase in FWC ( $p < 0.05$ ) when compared to NaCl (FWC = 0.429 g ice/g d.b). It is hypothesized that this increase in FWC relative to NaCl is due to stronger water-ion interactions.

In the case of doughs prepared with the Pembina, the FWC was found to be more sensitive to the alternative salt-types relative to NaCl than those prepared with Harvest (Figure 5.6). Pembina showed an increase in the FWC for all salt-types relative to NaCl ( $p < 0.01$ ), whereas this was not the case for Harvest. For doughs prepared with Harvest only MgCl<sub>2</sub> ( $p < 0.05$ ) and MgSO<sub>4</sub> ( $p < 0.01$ ) significantly increased in the amount of FWC relative to NaCl,

whereas all other alternative salts had similar FWC compared to NaCl ( $p>0.05$ ). However the effect of salt-type was less dramatic than that seen for cultivar differences. The difference in FWC between the stronger cultivar Pembina and the weaker cultivar Harvest is presumed to be associated with a weaker gluten network leaving more water available for freezing (i.e., Harvest is thought to have less bound water than Pembina).

#### 5.4.4 Thermogravimetric analysis (TGA)

Thermogravimetric analysis was used to examine if there was an effect of salts from the lyotropic series on water's association with being either near the surface of the dough existing as diffusive water (*relating to free water that can easily diffuse out of the dough*) or entrapped water within the gluten network (*relating to water that is more strongly absorbed multilayer and bound water*) as compared to NaCl. The first peak of the derivative thermogram gives an indication of water that is weakly interacting with the gluten network and is considered a part of the diffusive water layer. The second peak of the derivative thermogram is in accordance with the water that is more tightly associated with the gluten network either entrapped within the network as absorbed multilayer water or bound by the gluten proteins. Typical curves of the derivative thermogram for the dough samples used in this study can be seen in Figure 5.7A and 5.7B. A two-way analysis of variance found no significant effect of salt-type, flour cultivar, or the interaction salt-type and flour cultivar ( $p>0.05$ ) on weight loss of the first peak (Table 5.2). A two-way analysis of variance revealed that there was no significant effect of flour cultivar or salt-type ( $p>0.05$ ) on the first peak maximum temperature upon which the diffusive water layer was released (Table 5.2). Curve fitting was utilized to try to explain these unexpected results.

When examining the second peak maximum weight loss which relates to entrapped (more strongly absorbed multilayer water ) and bound water, a two-way analysis of variance was run and showed the main effects of salt and flour cultivar to not be significant ( $p>0.05$ ). However, there was one difference between the flour cultivars for NaCl, Harvest experienced a greater water loss at the second peak (~69%) than Pembina (~62%) ( $p<0.05$ ) (Table 5.2). This was unexpected given that Pembina forms a stronger gluten network than Harvest, however it is presumed to be due to Harvest containing more water in the dough formulation.

For the second peak maximum temperature at which the entrapped (more strongly absorbed multilayer water) and bound water was released, a two-way analysis of variance

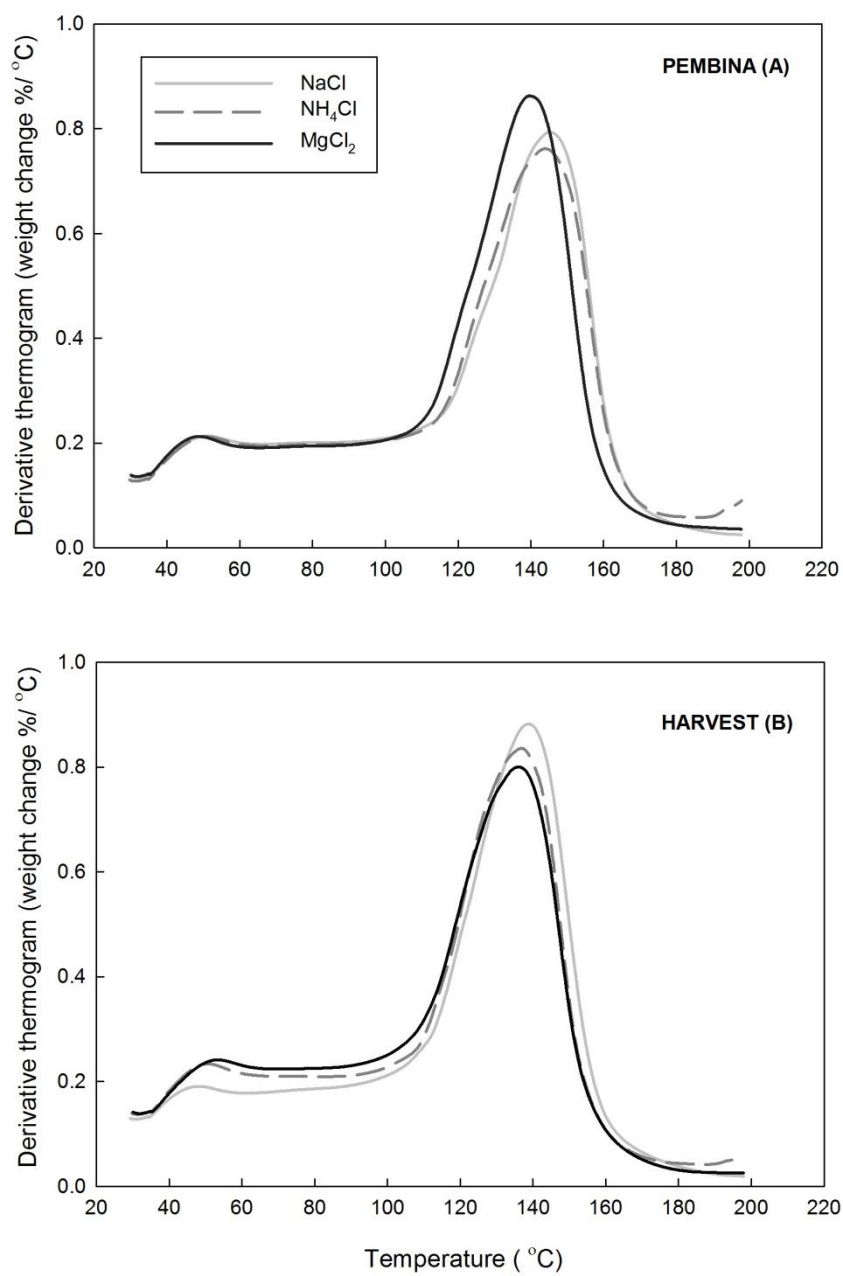


Figure 5.7 Derivative of the thermogram (weight change %/ °C) of doughs prepared with Pembina (A) and Harvest (B) as a function of salt-type (NH<sub>4</sub>Cl, NaCl, and MgCl<sub>2</sub>).

Table 5.2 Thermogravimetric analysis values for dough prepared using Pembina and Harvest in the presence of various salts from the lyotropic series (NH<sub>4</sub>Cl, NaCl, and MgCl<sub>2</sub>) at the 1% level (based on wt. flour). Data represents the mean  $\pm$  one standard deviation (n = 2).

Flour cultivar	Salt-type	1 <sup>st</sup> peak		2 <sup>nd</sup> peak		Total weight loss (%) <sup>2</sup>
		Max. peak height	Percent of total	Max. peak height	Percent of total	
		temp (°C)	weight loss (%) <sup>1</sup>	temp (°C)	weight loss (%) <sup>1</sup>	
Pembina	NH <sub>4</sub> Cl	49.66 $\pm$ 1.51 <sup>a</sup>	9.96 $\pm$ 0.20 <sup>a</sup>	144.50 $\pm$ 0.83 <sup>a</sup>	63.41 $\pm$ 3.26 <sup>a</sup>	45.15 $\pm$ 0.02 <sup>a</sup>
	NaCl	50.80 $\pm$ 0.24 <sup>a</sup>	10.51 $\pm$ 0.66 <sup>a</sup>	144.54 $\pm$ 0.99 <sup>a</sup>	61.89 $\pm$ 0.02 <sup>a</sup>	44.65 $\pm$ 0.12 <sup>a</sup>
	MgCl <sub>2</sub>	48.83 $\pm$ 0.04 <sup>a</sup>	10.05 $\pm$ 0.36 <sup>a</sup>	139.42 $\pm$ 0.38 <sup>b</sup>	64.68 $\pm$ 1.54 <sup>a</sup>	45.26 $\pm$ 0.05 <sup>a</sup>
Harvest	NH <sub>4</sub> Cl	50.22 $\pm$ 0.60 <sup>a</sup>	9.27 $\pm$ 0.08 <sup>a</sup>	136.82 $\pm$ 0.11 <sup>a</sup>	63.99 $\pm$ 1.06 <sup>a</sup>	45.66 $\pm$ 0.54 <sup>a</sup>
	NaCl	48.56 $\pm$ 0.63 <sup>a</sup>	9.07 $\pm$ 1.55 <sup>a</sup>	138.99 $\pm$ 0.31 <sup>b</sup>	69.31 $\pm$ 4.29 <sup>a</sup>	45.40 $\pm$ 0.22 <sup>a</sup>
	MgCl <sub>2</sub>	51.50 $\pm$ 2.57 <sup>a</sup>	11.11 $\pm$ 0.57 <sup>a</sup>	135.71 $\pm$ 0.52 <sup>a</sup>	66.03 $\pm$ 2.01 <sup>a</sup>	45.75 $\pm$ 0.13 <sup>a</sup>

Different letters for each flour cultivar in each column are significantly different (p<0.05).

<sup>1</sup>Percent of total weight loss % at the first and second peak was calculated by over laying the thermogram of weight % curve over the derivative weight change (weight change % /°C) curve and subtracting the end limit of the peak by the onset limit of the peak to obtain weight loss % over the peak interval. This value was then divided by the total weight loss % and multiplied by 100 to get the percent of total weight loss.

<sup>2</sup>Total weight loss (%) was calculated by: [(initial weight mg – final weight mg)/ initial weight mg] x 100.

showed that the main effects of flour cultivar and salt-type were significant ( $p < 0.001$ ), along with their two-way interaction ( $p < 0.05$ ). When examining the effect of salt-type (independent of flour cultivar), doughs prepared with NaCl required a slightly higher temperature ( $\sim 142^{\circ}\text{C}$ ) than  $\text{NH}_4\text{Cl}$  ( $\sim 141^{\circ}\text{C}$ ) meaning NaCl required slightly more energy necessary to release the entrapped and bound water, whereas  $\text{MgCl}_2$  required less energy ( $\sim 138^{\circ}\text{C}$   $p < 0.001$ ) (Table 5.2). Therefore,  $\text{MgCl}_2$  takes less energy to release that water, therefore it is not as strongly absorbed or bound as it is with  $\text{NH}_4\text{Cl}$  and NaCl. Overall when comparing flour cultivars (independent of salt-type) Pembina required a greater temperature ( $\sim 143^{\circ}\text{C}$ ) than Harvest ( $\sim 137^{\circ}\text{C}$ ) ( $p < 0.001$ ), this is potentially indicative of Pembina forming a stronger gluten network than Harvest and therefore requiring more energy to release the water from the stronger formed gluten network. For every salt-type, Pembina required a greater temperature than Harvest to release the entrapped and bound water ( $p < 0.01$ ).

When examining the total amount of water lost, a two-way analysis of variance revealed that only the main effect of flour cultivar was significant ( $p < 0.01$ ) and the main effect of salt-type and their interaction was not significant ( $p > 0.05$ ). Pembina experienced less total water loss (45.02%) than Harvest (45.60%) ( $p < 0.01$ ). It is believed that the greater total water lost by Harvest than Pembina is due to the fact that Harvest doughs are prepared with greater water addition than Pembina.

#### 5.4.4.1 Thermogravimetric analysis (Deconvolution of the derivative thermogram [DTG])

A deconvolution algorithm of the derivative of the TGA thermogram was performed similar to Roozendaal et al. (2012) for all doughs prepared with Pembina and Harvest at the 1% salt level using salts from the lyotropic series representing two stabilizing salts and one destabilizing salt ( $\text{NH}_4\text{Cl}$ , NaCl, and  $\text{MgCl}_2$ ) to give an indication of the different water states within the doughs. Roozendaal et al. (2012) used a 5 peak model to indicate free water (peak 1), starch (mobile and bound water [peak 2 & 3]), and gluten (mobile and bound water [peak 4 & 5]); however, it was found for our samples that a 6 peak model produced a higher agreement with the DTG  $r^2$  fit. The peaks represent the water associated with either free water (peak 1 and 2), starch unbound and bound water (peak 3 and 4, respectively), and gluten unbound and bound water (peak 5 and 6, respectively) which can be observed in Figure 5.8A & B. Water loss for each peak and peak temperature for water are given in Table 5.3. In Table 5.3 it appears that for

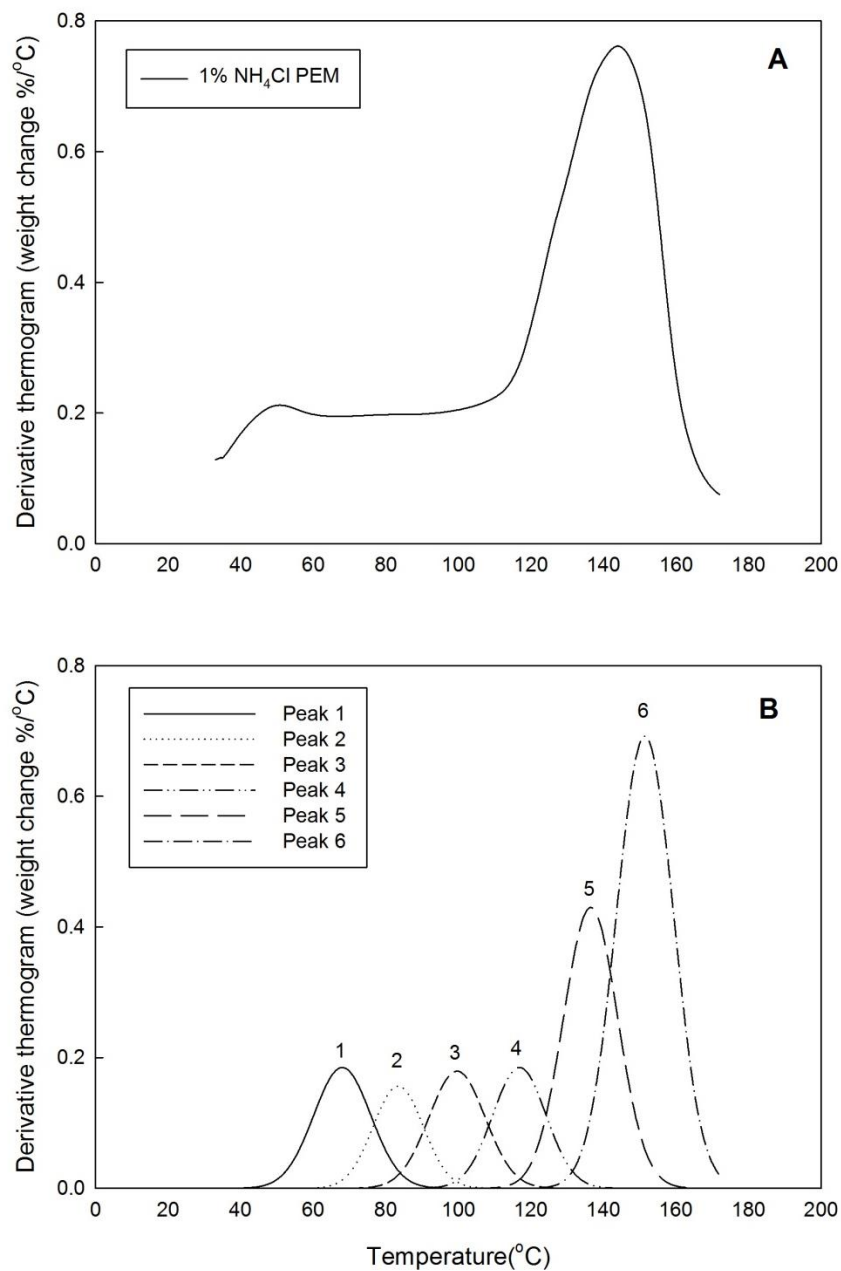


Figure 5.8 Derivative thermogram (weight change %/°C) for a dough prepared with Pembina at 1%  $\text{NH}_4\text{Cl}$  (A) and the deconvolution of the derivative thermogram (B). Peak 1 & 2 represents water associated with free water, peak 3 & 4 represents water associated with starch both mobile and bound respectively, and peak 5 & 6 represent water associated with gluten both mobile and bound respectively.

Table 5.3 Peak fitting data for peak temperature and relative proportion of peak water loss for doughs prepared using Pembina and Harvest as a function of salts from the lyotropic series (1%  $\text{NH}_4\text{Cl}$ ,  $\text{NaCl}$  and  $\text{MgCl}_2$ ). Values represent means samples  $\pm$  one standard deviation (n=2).

Flour Cultivar	Water's Association	Peak Number	Peak Temperature ( $^{\circ}\text{C}$ )			Relative Proportion of Peak Water Loss (%)		
			$\text{NH}_4\text{Cl}$	$\text{NaCl}$	$\text{MgCl}_2$	$\text{NH}_4\text{Cl}$	$\text{NaCl}$	$\text{MgCl}_2$
Pembina	Free	1	$44.3 \pm 0.0$	$45.6 \pm 0.4$	$41.6 \pm 0.4$	$10.2 \pm 0.4$	$10.1 \pm 1.3$	$6.2 \pm 0.1$
		2	$62.9 \pm 0.4$	$66.0 \pm 0.3$	$56.3 \pm 0.5$	$7.3 \pm 0.6$	$10.4 \pm 1.5$	$9.1 \pm 0.4$
	Starch	3	$83.3 \pm 0.2$	$89.8 \pm 0.2$	$78.5 \pm 0.5$	$10.2 \pm 0.2$	$11.2 \pm 0.1$	$10.8 \pm 1.9$
		4	$104.2 \pm 0.1$	$112.1 \pm 2.2$	$102.5 \pm 0.7$	$8.9 \pm 1.3$	$10.9 \pm 1.9$	$10.4 \pm 0.9$
	Gluten	5	$128.8 \pm 0.5$	$134.1 \pm 2.2$	$126.8 \pm 0.1$	$24.2 \pm 1.4$	$26.8 \pm 1.5$	$26.7 \pm 0.2$
		6	$147.0 \pm 0.5$	$148.7 \pm 1.0$	$142.8 \pm 0.7$	$39.2 \pm 0.6$	$30.6 \pm 3.5$	$36.8 \pm 0.7$
Harvest	Free	1	$41.8 \pm 0.5$	$39.8 \pm 0.1$	$40.4 \pm 0.5$	$8.8 \pm 0.8$	$7.2 \pm 0.5$	$7.4 \pm 0.1$
		2	$60.4 \pm 0.5$	$55.3 \pm 0.3$	$56.4 \pm 0.4$	$10.6 \pm 0.9$	$6.7 \pm 0.2$	$8.9 \pm 2.1$
	Starch	3	$80.6 \pm 0.1$	$72.3 \pm 0.2$	$73.7 \pm 2.3$	$8.2 \pm 0.3$	$6.7 \pm 0.3$	$7.5 \pm 0.8$
		4	$100.4 \pm 0.6$	$97.0 \pm 0.0$	$96.5 \pm 0.9$	$10.4 \pm 1.5$	$17.0 \pm 1.3$	$16.6 \pm 2.2$
	Gluten	5	$122.6 \pm 2.6$	$127.3 \pm 0.8$	$124.0 \pm 0.0$	$24.1 \pm 7.3$	$28.5 \pm 2.9$	$29.0 \pm 0.3$
		6	$139.2 \pm 1.4$	$143.1 \pm 1.1$	$140.2 \pm 0.5$	$37.8 \pm 7.8$	$33.9 \pm 0.6$	$30.6 \pm 0.3$

Pembina,  $\text{NH}_4\text{Cl}$  has the closest effect to  $\text{NaCl}$  when comparing peak temperatures whereas  $\text{MgCl}_2$  appears to result in a decrease of peak temperature, presumably due to the destabilizing effect of  $\text{MgCl}_2$  on the gluten network and thus resulting in less energy needed to remove the water. There does not appear to be a clear trend when comparing peak water loss of  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  to  $\text{NaCl}$ . However, it is interesting to note that for the gluten peak 6 (bound water) there was an increase in bound water with  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  when compared to  $\text{NaCl}$ . Water loss for the doughs prepared with  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  compared to  $\text{NaCl}$  seem to be fairly similar for each peak except for the free water peaks and the gluten peak 6 (bound water). It appears that with the alternative salts that there is less water lost from the free water peaks and more for the gluten network when compared to  $\text{NaCl}$ .

When examining the peak temperatures for free water and starch for doughs prepared with Harvest, it can be seen that the alternative salt  $\text{NH}_4\text{Cl}$  increases the temperature compared with  $\text{NaCl}$ , whereas the alternative salt of  $\text{MgCl}_2$  has similar temperatures to  $\text{NaCl}$ . However, for peak temperatures for the gluten peaks it appears that  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  decreased the peak temperature at which water was lost from the gluten when compared to  $\text{NaCl}$ . When looking at water loss for the alternative salts compared to  $\text{NaCl}$  it appears that the greatest difference in water loss is for the starch peak 4 where  $\text{NH}_4\text{Cl}$  is lower than that of both  $\text{NaCl}$  and  $\text{MgCl}_2$  whereas all other peak water loss fractions appear to be fairly similar.

A two-way ANOVA was carried out on water loss for the free water (peak 1) to find that the main effects of salt-type ( $p < 0.01$ ) and flour cultivar ( $p < 0.05$ ), along with their associated interaction ( $p < 0.05$ ) to be significant, however, for free water from peak 2 only the interaction term was significant ( $p < 0.05$ ). For the starch-fraction peaks, it found that for peak 3, the main effect of salt-type and interaction term was not significant ( $p > 0.05$ ), however flour cultivar was significant ( $p < 0.01$ ). For peak 4 of the starch-fraction, the main effects of salt-type ( $p < 0.05$ ) and flour cultivar ( $p < 0.01$ ) were significant, however their interaction was not ( $p > 0.05$ ). For both gluten-fraction peaks (peaks 5 and 6), both main effects and their interaction were not significant ( $p > 0.05$ ). It appears that the addition of alternative salts had a similar effect as  $\text{NaCl}$  in study 2, where the greatest effect was found on the free water and starch peaks for water loss.

For purposes of simplification, discussions will focus on differences after adding the two peaks associated with free water (peaks 1+2), the starch-fraction (peaks 3+4), and the gluten-fraction (peaks 5+6), as shown in Figure 5.9A-C for doughs prepared with Pembina and Harvest



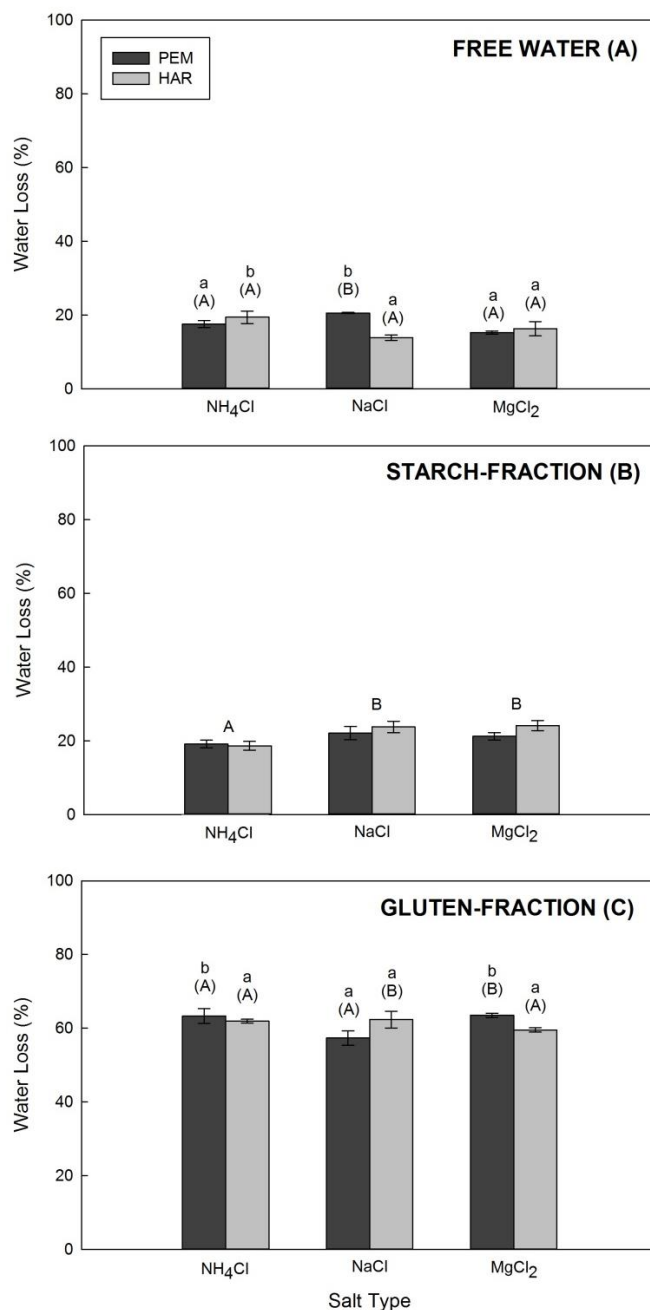


Figure 5.9 Peak fitting data representing total peak free water (A), starch-fraction (B), and gluten-fraction (C) water loss (%) for doughs prepared using Pembina (PEM) and Harvest (HAR) as a function of salts from the lyotropic series (1% NH<sub>4</sub>Cl, NaCl, MgCl<sub>2</sub>). Values represent means  $\pm$  sd. (n=2). Lower case letters represent comparisons within a flour cultivar between salt-type. Upper case letters represent comparisons between flour cultivars for each salt-type. Different levels are significantly different  $p < 0.05$ . \*NOTE: Figure B uppercase letters represent comparison between the main effect of salt-type regardless of flour cultivar.

Table 5.4 Probability values arising from a two-way analysis of variance for peak fitting data for proportional area under the curve for free water peaks, starch water peaks and gluten water peaks for doughs prepared using CWRS wheat cultivars (Pembina [PEM] and Harvest [HAR]) as a function of salts from the lyotropic series (1%  $\text{NH}_4\text{Cl}$ ,  $\text{NaCl}$ ,  $\text{MgCl}_2$ ).

	Peak area		
	Free water	Starch-fraction	Gluten-fraction
	(Peaks 1 + 2)	(Peaks 3 + 4)	(Peaks 5 + 6)
Main effects			
Salt-type	NS	$p < 0.01$	NS
Flour cultivar	NS	NS	NS
Interaction			
Salt-type*flour cultivar	$p < 0.01$	NS	$p < 0.05$

flours. A two-way ANOVA of this data is given in Table 5.4. For free water, the main effects of salt-type and flour cultivar were not significant ( $p > 0.05$ ), however their interaction was ( $p < 0.01$ ). For doughs prepared with Pembina it was observed that the loss of free water was similar regardless of the alternative salt-type. However, in the case of Harvest, the presence of  $\text{NH}_4\text{Cl}$  resulted in a higher amount of water loss, whereas doughs with  $\text{NaCl}$  and  $\text{MgCl}_2$  were similar (Figure 5.9A).

In Figure 5.9B water association with the starch-fraction is presented and a two-way ANOVA found only salt-type to be significant ( $p < 0.01$ ), whereas flour cultivar and their interaction was not ( $p > 0.05$ ). It can be seen that doughs prepared with the alternative salt  $\text{NH}_4\text{Cl}$  have less water associated with the starch-fraction (~19%) relative to doughs prepared with  $\text{NaCl}$  and  $\text{MgCl}_2$  which have significantly more (~23%). In the case of the gluten-fraction, its water association is shown in Figure 5.9C. A two-way ANOVA found only the interaction term between salt-type and flour cultivar to be weakly significant ( $p < 0.05$ ), however the main effects

alone were not significant ( $p>0.05$ ). For Harvest, the amount of water associated with the gluten-fraction was similar regardless of the salt, whereas in the case of doughs prepared with Pembina,  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  resulted in an increase in the amount of water associated with the gluten relative to  $\text{NaCl}$ . Which is opposite of the effect of the alternative salts on Pembina's free water when comparing to  $\text{NaCl}$ . The free water appears to migrate to the gluten-fraction with the addition of the alternative salts  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  when compared to  $\text{NaCl}$  for Pembina. This trend was not noticed for doughs prepared with Harvest, where the free water increased with  $\text{NH}_4\text{Cl}$  relative to  $\text{NaCl}$  and  $\text{MgCl}_2$ , which were similar. This resulted in a decrease in water associated with the starch-fraction whereas  $\text{NaCl}$  and  $\text{MgCl}_2$  resulted in an increase. It appears that the alternative salts for the Pembina have an effect of water movement between free and gluten-fraction whereas alternative salts for Harvest have an effect of water movement between free and starch-fraction. The differences in the effect of salt-type on water migration in the two cultivars could be partly explained by differences in flour composition and quality, more specifically the differences in gluten protein quality and the amount of damaged starch.

#### **5.4.5 Confocal laser scanning microscopy (CLSM)**

Confocal laser scanning micrographs of doughs prepared with both Pembina and Harvest flours as a function of salt-type and level (1 vs 2%) are given in Figures 5.10 and 5.11, respectively. In the case of doughs prepared with Pembina, no substantial differences were observed in the morphologies of the dough regardless of the salt-type (Figure 5.10). However, micrographs hint at some slight differences with the addition of  $\text{NH}_4\text{Cl}$  resulting in a more continuous and extended fibril gluten network structure than those with the addition of  $\text{NaCl}$  (Figure 5.10C-D & 5.10A-B). In contrast dough prepared with  $\text{MgCl}_2$ , had gluten networks with fewer fibres that appeared discontinuous with more aggregates than those prepared with  $\text{NaCl}$  (Figure 5.10E-F & 5.10A-B). It is presumed that the more ordered gluten network (i.e., with  $\text{NH}_4\text{Cl}$  present) resulted from higher amounts of cohesive forces, leading to less sticky dough (Figure 5.5). The amount of cohesive forces is then hypothesized to decrease creating a network with less ordered gluten polymers (i.e., with  $\text{MgCl}_2$ ) leading to increased stickiness (Figure 5.5). All doughs showed unidirectional ordering of the gluten polymers.

In the case of doughs prepared with Harvest, no substantial differences were observed in the morphologies of the dough regardless of the salt level within each salt-type (Figure 5.11).

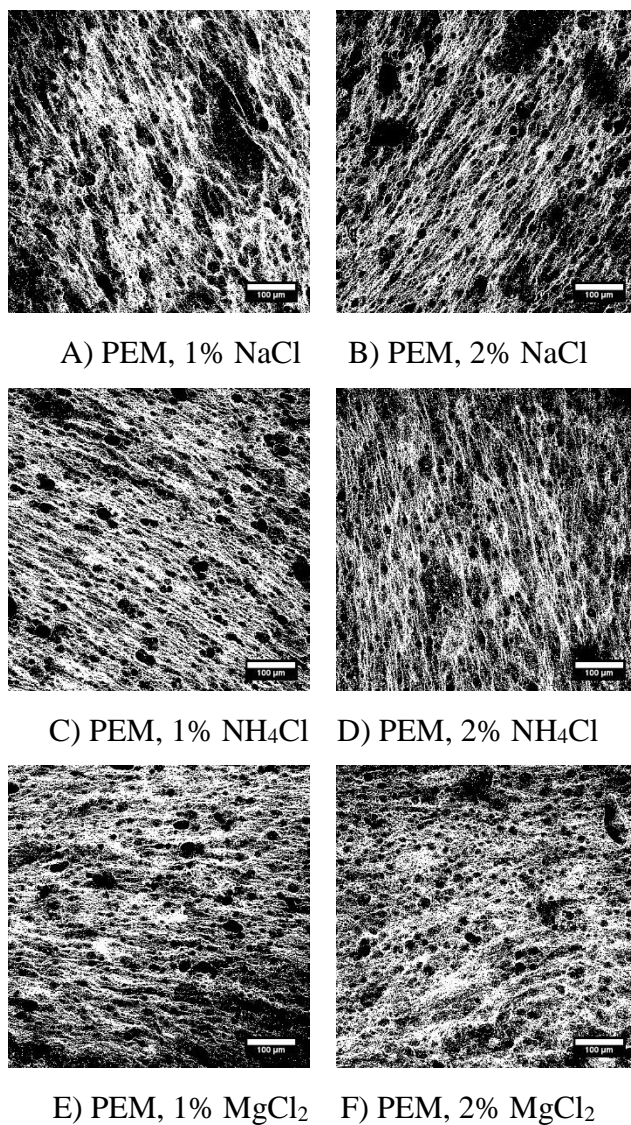


Figure 5.10 Confocal laser scanning microscopy images for dough prepared using Pembina (PEM) in the presence of various salts from the lyotropic series (NaCl, NH<sub>4</sub>Cl, and MgCl<sub>2</sub>) at the 1 and 2% level (based on flour weight).

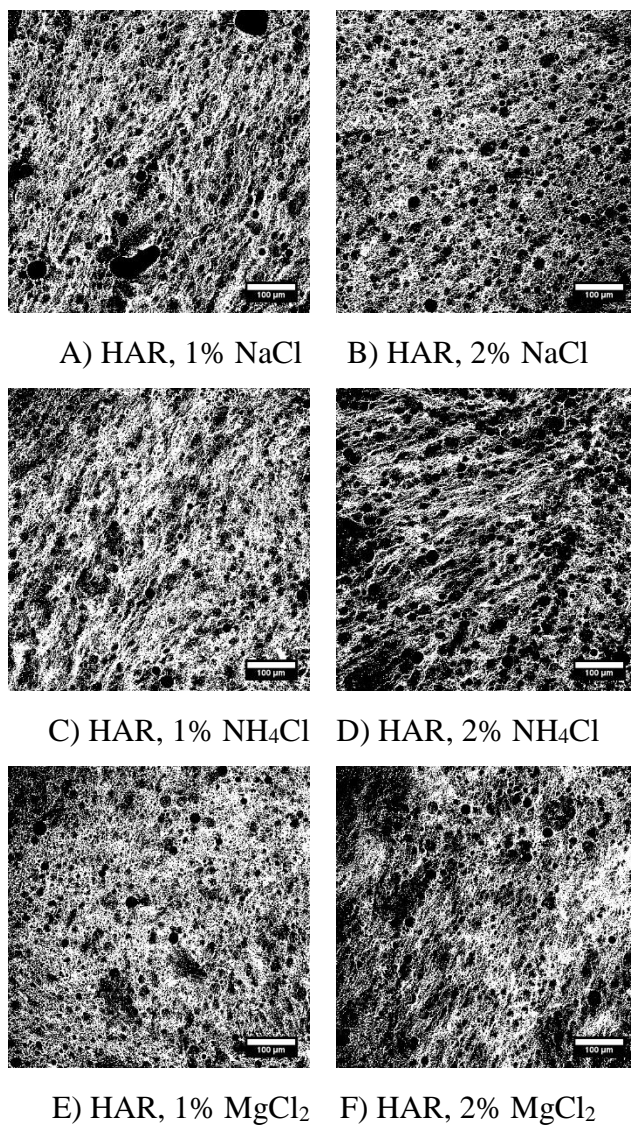


Figure 5.11 Confocal laser scanning microscopy images for dough prepared using Harvest (HAR) in the presence of various salts from the lyotropic series (NaCl, NH<sub>4</sub>Cl, and MgCl<sub>2</sub>) at the 1 and 2% level (based on flour weight).

However, micrographs hint at some slight differences with dough prepared in the presence of  $\text{NH}_4\text{Cl}$  showing greater ordering of extended fibril gluten polymers relative to doughs prepared with  $\text{NaCl}$ . In the case of the latter, the morphology appeared more porous with multi-directional ordering of shorter polymers and aggregates. The difference in morphology between doughs prepared with  $\text{NaCl}$  relative to  $\text{NH}_4\text{Cl}$  also corresponded to a decrease in stickiness (Figure 5.5). However in contrast to Pembina, the addition of  $\text{MgCl}_2$  to dough prepared with Harvest showed improved directional ordering of the gluten polymers relative to doughs prepared with  $\text{NaCl}$ . The increased ordering with  $\text{MgCl}_2$ , combined with the similar migration of water as  $\text{NaCl}$  to the starch-fraction from free water (Figure 5.9), was postulated to have contributed to the decreased stickiness within the dough relative to those with  $\text{NaCl}$  (Figure 5.5).

Tuhumury and others (2016a) also examined the effect of salts from the lyotropic series on the gluten network formation using confocal laser scanning microscopy. They stated that a fibrous strand-like interconnected gluten microstructure was formed with the use of the non-chaotropic cations ( $\text{NH}_4^+$ ,  $\text{Na}^+$ ), whereas the chaotropic cation ( $\text{Mg}^{2+}$ ) resulted in the formation of a honeycomb like gluten microstructure. They described doughs with  $\text{NaCl}$  as more fibrous and extended, whereas doughs prepared with  $\text{NH}_4\text{Cl}$  were described as having fewer observable fibrous structures. This observation was the opposite of what was seen in the current study, where  $\text{NH}_4\text{Cl}$  at the 2% level was found to improve the gluten network microstructure for both flour cultivars (Pembina and Harvest). The differences in findings between this study and Tuhumury and others, with the use of  $\text{NH}_4\text{Cl}$ , could be due to the use of different mixer types, mixing times, method of salt addition, and different flour cultivars. The use of different flour cultivars with differing compositions are believed to have an impact as it was found in research done by Butow and others (2002) that different flour cultivars with differing glutenin compositions had differing degrees of sensitivity to changes in salts with regard to their dough handling properties. However, similar findings between Tuhumury and others (2016a) and the current study, with the use of the flour cultivar Pembina, were found with the use of  $\text{MgCl}_2$ . Tuhumury described the effect of  $\text{MgCl}_2$  on the gluten network formation as creating gluten that formed large aggregates that appeared homogeneous and continuous. However, it appeared that the network formation found in Tuhumury was more negatively impacted by the use of  $\text{MgCl}_2$  than in the present study for doughs prepared with Pembina. On the other hand, in the current study the flour cultivar Harvest noticed an improvement in the gluten network at the 2% level,

which was postulated to be due to the effect of different flour cultivars' glutenin compositions and thus sensitivity to salts (Butow et al., 2002). Tuhumury and others (2016a) state that the formation of the more fibrous stranded gluten networks for doughs prepared with monovalent cation salts ( $\text{NH}_4\text{Cl}$  and  $\text{NaCl}$ ) is believed to be due to enhanced hydrogen bonding within the glutenin molecules as a result of the competition of monovalent cations with water.

## 5.5 CONCLUSIONS

Throughout the analysis, regardless of salt-types, doughs prepared with Pembina formed stronger gluten networks than doughs prepared with Harvest as indicated by the rheological results. Between the two flour cultivars, doughs prepared with Harvest appeared to be more sensitive to the salt-type used as indicated by the variability in strength of the gluten network. It was expected that if a salt-type increased the dough strength then dough stickiness would decrease; on the other hand if the salt-type decreased the dough strength then dough stickiness would increase. Pembina consistently had lower dough stickiness than Harvest in all cases because it consistently formed a stronger gluten network, however the extent of the change in dough stickiness was influenced by the different salt-types. Interestingly the trend in dough stickiness did not necessarily follow the trend seen in the rheological results with the effect of the salt-type on the gluten network strength. Magnesium chloride which was found to have a weakening effect on both flour cultivars' gluten network strength, actually resulted in a decrease or no significant difference in the dough stickiness depending on the level when compared to the  $\text{NaCl}$  whereas the opposite result was expected. Ammonium chloride was found to increase the gluten network strength for both flour cultivars, with the effect being greater for Harvest,  $\text{NH}_4\text{Cl}$  also resulted in a decrease in the dough stickiness.

The stronger gluten network of Pembina was found to mitigate some of the effects of reducing salt levels compared to Harvest. In all cases,  $\text{NH}_4\text{Cl}$  was found to be most effective at reduced salt levels at strengthening the gluten-gluten interactions, most likely through hydrophobic interactions as protein-protein interactions become favoured over protein-water. Ammonium chloride was found to decrease dough stickiness the most compared to  $\text{NaCl}$  at the low level for both the strong and weaker dough producing flours and it also showed the greatest strengthening effects, showing that  $\text{NH}_4\text{Cl}$  could be a potential replacement for  $\text{NaCl}$  at the low level of 1% inclusion. Less may even be needed to provide a similar effect as  $\text{NaCl}$  in the

stronger dough producing flour cultivars. However baking trials and sensory analysis would be necessary to examine loaf volume, crumb structure and texture and loaf colour and flavor. Further analysis on partial replacement of NaCl with  $\text{NH}_4\text{Cl}$  should be examined as well.

The differing effect of the salt-types on dough strength and stickiness for the flour cultivars points to another factor affecting the occurrence of stickiness with the use of salt replacements at both the low and high level. This could be due to the effect of the salt-types on the water association within the dough being either with the starch or the gluten as opposed to free. For the stronger dough producing flour cultivar of Pembina the alternative salts had more of an effect on the water's movement between free and the gluten-fraction. For Harvest the alternative salts have more of an effect on water movement between free water and the starch-fraction. These findings lead to the conclusion that the gluten network may not be the only determinant in dough stickiness in weaker flour cultivars; instead it may also be the movement of water between the free water and the starch-fraction. Further support for this conclusion comes from the fact that for the weaker flour cultivar of Harvest both the stabilizing and destabilizing salts improved the gluten network formation seen in the confocal laser scanning microscopy images compared to NaCl. Dough stickiness was also decreased comparatively whereas the rheological results relating to dough strength indicate a weakening effect of the destabilizing salt. Future studies should be done using both NMR and attenuated total reflectance and Fourier transform infrared spectroscopy method to examine alternative salts that will achieve the same movement of water from free to the starch-fraction as NaCl in weaker flour cultivars to allow for replacement and prevent dough stickiness. In other studies researchers have found that up to 50% replacement of NaCl with KCl has been done with fair results, however flavour was still an issue. Thus in future studies 50%  $\text{NH}_4\text{Cl}$  could be used to replace 50% of NaCl and flavour tested because some of the ammonium would be given off as volatiles during baking and thus the flavour of the bread may not be affected.



## **6. GENERAL DISCUSSION**

### **6.1 Overview**

Sodium reduction for dough formulations can result in a significant increase in stickiness, which causes costly processing delays and quality issues within the dough and the final loaf quality. As such, the overall goal of this research was to gain a better understanding of the role of sodium in controlling dough handling properties at normal and reduced salt (NaCl) levels, and then to develop strategies to help combat the occurrence of dough stickiness. The research was divided into three main studies that focus on: a) the impact of cultivar composition on dough rheology; b) the impact of NaCl reduction in controlling dough rheology, stickiness and water mobility; and c) the role of alternative salts in controlling dough rheology, stickiness and water mobility.

### **6.2 Cultivar selection**

For this research, flour cultivars were chosen based on a historical trial involving 37 different cultivars that spanned 69 years of development and registrations, all within the wheat class: Canada Western Red Spring (CWRS) (Yovchev et al., 2017). CWRS wheat is described as hard wheat having superior milling and baking qualities, primarily used in pan bread production, but also in hearth bread, steamed bread, noodles, flat bread and common wheat pasta (Edwards et al., 2012). Overall, in a span of 69 years, breeding programs have bred for increased yield and disease resistance, while maintaining good dough handling properties and bread quality at the industry standard NaCl level of 2%. Recently though, increased awareness of linkages between dietary sodium consumption and the risk for hypertension, cardiovascular disease and stroke has driven governmental regulators and the baking industry to look at low salt formulations. However, within the low sodium environment, cultivars that performed well in terms of yield and disease resistance now are unable to maintain quality standards in terms of dough handling and bread quality. The study by Yovchev et al. (2017) was aimed at re-evaluating 37 different cultivars along the breeding history for dough handling and baking quality within a normal and

low salt environment. The four cultivars chosen in this present study reflect dough that displayed good handling properties under normal NaCl conditions (2%), however under reduced salt conditions (1.1%), cultivars displayed good (Pembina, Roblin), intermediate (McKenzie) and poor (Harvest) handling. Stickiness was also found to increase with salt reduction for all cultivars, however in a greater extent with the dough displaying intermediate and poor handling properties (Yovchev et al., 2017). A greater understanding of the compositional differences between the selected cultivars may shed light into mechanisms governing differences in their dough handling and stickiness. Results from this work may help breeders make linkages to past breeding practices during new cultivar development that may have resulted in these compositional changes.

Pembina was developed in 1948 involving a cross between three wheat cultivars (Thatcher x McMurachy-Exchange x Redman), and then was later registered in 1959 (Campbell, 1963). Pembina showed exceptional baking quality and gluten strength in comparison to other cultivars at the time (Campbell, 1963). Its properties weren't surpassed until the registration of Roblin ~30 y later. Roblin is high in protein, and was developed in 1976 by wheat cultivars from four pedigrees: RL4302, RL4356, RL4359 and RL4353 (Campbell & Czarnecki, 1987). McKenzie was the first doubled haploid wheat cultivar developed in Canada from the first generation offspring from the parent cultivars Columbus and Amidon in 1989, and later registered in 1997 (Graf et al., 2003). McKenzie has been shown to meet the protein content, milling properties, dough functionality and baking performance of check cultivars, such as Roblin. However, McKenzie has harder kernels and therefore higher damaged starch than the check cultivars (Graf et al., 2003). Harvest was produced from the cross between AC Domain\*2/ND640 in 1991 and then later given registration in 2002 (Fox et al., 2010). Harvest has similar milling and baking performance to the check cultivars, and has similar grain hardness and starch damage as McKenzie (Fox et al., 2010).

An understanding of the structure-function relationships controlling dough rheology and stickiness involving flours with inherent compositional and quality differences will help to develop strategies for combating dough stickiness within a reduced sodium environment.

### **6.3 Impact of different CWRS wheat cultivars and their composition on the handling of dough with normal NaCl formulations**

Lasztity and Abonyi (2009) and Goesaert et al. (2005) indicated differences among cultivars in terms of their elasticity and extensibility are related to their chemical composition [gluten proteins (quantity/quality, ratio of glutenins to gliadins, ratio of high to low molecular weight glutenins, and amino acid content)], starch (damaged/native), non-starch polysaccharides, and other ingredients within the dough formulation (water, salt, yeast, sugar, etc.).

#### *a) Impact of protein content and quality on dough rheology*

The protein content, on a 14% moisture basis, within the flour plays an important role in water absorption and gluten strength within in the dough, which in turn impacts its ability to form a low density loaf of bread with a fine and uniform crumb (Lai & Li, 2006). Dobraszczyk and Salmonowicz (2008) found that protein content and protein quality work independently of each other, and gluten content alone as predictor of baking quality would not give adequate results. Uthayakumaran and Lukow (2003) examined the rheological properties (e.g., mixograph and extensigraph) of doughs prepared using flours from a range of wheat cultivars grown in Canada. The authors found that cultivars with higher protein levels did not necessarily show the greatest dough strength, where it was concluded that strength was related to both the protein content, and differences in the glutenin: gliadin ratios and the LMW-glutenin composition. Others have also suggested a link between dough strength and both protein content and quality (Gupta et al., 1992; Khatkar et al., 1995, 1996; Wang & Sun, 2002; Sliwinski et al., 2004; Dobraszczyk & Salmonowicz, 2008; Barak et al., 2013; McCann & Day, 2013).

In the present study, Roblin contained the highest protein content (~16.2% d.b.), followed by McKenzie and Harvest (~15.1% d.b.), which were similar, and then Pembina (14.7% d.b.) (Table 6.1). However, an examination of the empirical rheological properties of the dough indicated that strength followed a different trend suggesting that protein content alone was not governing dough strength. For example, both resistance to extension (measured by large deformation testing – extensigraph) and work input to peak development (measured using the mixograph) data for each cultivar showed a similar trend, where Pembina formed the strongest dough, followed by Roblin and McKenzie, which were similar and then Harvest.

Table 6.1. Gives the protein and damaged starch levels, farinograph water absorption (FAB), as well as both raw and normalized work input to peak development (mixograph testing) and resistance to extension (extensigraph testing) data for four different CWRS cultivars. All rheological data was normalized to the FAB value of Pembina.

Cultivar	Protein content (% d.b.)	Damaged starch (%)	FAB (% to 14% w.b.)	Work input to peak development		Resistance to extension (N)	
				(% tq. min)		Raw data	Normalized
				Raw data	Normalized		
Pembina	14.7 ± 0.0	6.0 ± 0.3	61.5 ± 0.3	117.9 ± 6.7	117.9 ± 6.7	0.31 ± 0.02	0.31 ± 0.02
Roblin	16.2 ± 0.0	5.5 ± 0.1	65.3 ± 0.1	103.7 ± 2.4	110.1 ± 2.6	0.25 ± 0.01	0.27 ± 0.01
McKenzie	15.1 ± 0.0	7.1 ± 0.2	63.3 ± 0.7	97.4 ± 2.7	100.3 ± 2.8	0.23 ± 0.00	0.23 ± 0.00
Harvest	15.1 ± 0.1	7.1 ± 0.2	64.9 ± 0.1	85.4 ± 5.6	90.1 ± 5.9	0.18 ± 0.01	0.19 ± 0.01

In terms of wheat quality data, both Pembina and Roblin showed similarly high gluten indices, whereas Roblin showed a greater gluten performance index than Pembina, both of which are related to the strength of the gluten network. In contrast, both gluten indices were lower for McKenzie and Harvest indicating that they would form weaker doughs. This supports the rheological results, where it was found the Pembina and Roblin formed stronger doughs. To probe the complexity of the inter-relationships between protein content, wheat quality and dough rheology further between cultivars, an examination of the protein composition was performed to help give greater insight to differences in the macroscopic viscoelastic behaviour of the dough prepared from different cultivars.

In general, studies in the literature have indicated that wheat cultivars with high glutenin: gliadin ratios lead to stronger gluten networks (Khatkar et al., 1995; Janssen et al., 1996; Barak et al., 2013). In the present study, Pembina and Roblin were found to have similarly high glutenin: gliadin ratios relative to McKenzie and Harvest helping to explain why Pembina and Roblin formed strong dough and, McKenzie and Harvest resulted in weaker dough. Differences within dough strength between the stronger cultivars, Pembina and Roblin are less clear, since Pembina formed stronger dough. Literature studies have also shown that cultivars with higher ratios of high (HMW): low (LMW) molecular weight glutenin subunits form stronger gluten

networks (Gupta et al., 1992; Sliwinski et al., 2004), since the larger polymers forms loops ( $\beta$ -turns) and trains ( $\beta$ -sheets) within the gluten network creating a large interconnected network with the ability to store elastic energy within the dough (Belton, 1999; Singh & MacRitchie, 2001; Belton, 2012). However, in the literature the role of LMW-GS's is poorly understood. In the present study, Roblin was found to have a higher HMW: LMW glutenin subunit (GS) ratio than Pembina, but had lower dough strength. As a result, the reasons why Pembina formed a stronger dough than Roblin still remains unclear, but are hypothesized to be caused by: a) differences in composition of the HMW-GS, which could lead to differences in the amount of loops and trains present and the subsequent physical entanglement within the gluten network (not evaluated in this research); and b) higher levels of LMW-GS proteins present in the Pembina flour which may act as cross-links, extenders or branch points to connect more of the HMW-GS polymers together to form a stronger more connected network.

McLeish and Larson (1998) developed a model to describe the non-linear extensional rheology of polymer melts (low density polyethylene) with long-chain branches and multiple junction zones that shows both strain hardening and shear thinning properties. The model describe a long chain branched polymer melt as having a single backbone with branches arising from each end. These branches (termed the pom poms) are entangled with surrounding polymers to form a large macrostructure. Under conditions of extensional flow, the backbones become stretched and display first strain hardening (e.g., rapid non-linear increase of viscosity with imposed strain), and then later strain softening (e.g., decrease in viscosity in response to imposed strain – shear thinning) as alignment of the polymer backbones increases. In 2003, Dobraszczyk & Morgenstern applied this model to high molecular weight branched gluten polymers (glutenin) within a dough system, whose branch points lead to entangled high ordered structures (gluten network) that would lead to strain hardening in doughs which would resist extension. Building off this model in the current study, it is hypothesized that the LMW-GS could act as: a) the 'pom poms' or chain terminators, which could lead to additional branch points; b) as chain extenders by having cysteines present in both the N and C terminal ends of its structure, enabling it to form inter-molecular cross-links within the gluten network to elongate HMW-GS or connect parallel glutenin polymers; and increased physical entanglement. These effects are thought to give rise to greater strain hardening within the dough, to increase its resistance to extension and strength.

*b) Impact of water content, protein content and damaged starch on dough strength*

Water within dough acts to hydrate the gluten proteins and acts as a plasticizer to increase mobility of the gluten network (Jekle & Becker, 2011). During dough mixing, each flour is mixed based on its farinograph water absorption until it reaches optimal dough development. Depending on the flour's composition (i.e., protein content and quality, starch and pentosans), the amount of water added to the dough formulation can vary (Eliasson & Larsson, 1993a; Tsilo et al., 2013; Meerts et al., 2017). If too little water is added to the flour the dough formed will lack cohesion, whereas flour with too much water will have a dough softening effect and will become weaker (Bloksma & Bushuk, 1988). The majority of studies in the literature either prepare dough using a range of water level additions such that FAB is adjusted to be below, at and above the optimal FAB for dough development. This approach is typically done when researchers are using one cultivar. For comparing multiple cultivars, typically researchers prepare dough to the FAB where optimal dough development occurs, as was the case in the present study. Even though there may be different water levels present, all doughs were mixed to their optimal dough development for comparative purposes. In order to display, the effect of different amounts of water added to the dough formulations in the present study, resistance to extension (from large deformation testing – extensigraph) and work input to peak dough development (WIP) (from mixograph testing) were normalized to the FAB content of Pembina (Table 6.1.). In doing so, WIP and resistance to extension values increased, closer to that of Pembina, since Pembina had the lowest FAB values, however the trend in the data remained unchanged relative to the un-normalized data (Table 6.1). Findings from the normalization procedure indicate that the different water levels added did not play a significant role at impacting dough strength, most likely since all doughs were mixed to the optimal development. It was seen in Wang and Sun (2002) that in some cases, cultivars having higher FAB values, did not result in a decrease in their resistance to extension data, relative to cultivars that had lower FAB values. Jekle and Becker (2011) reported if too much water is added to the flour beyond the optimal FAB amount for a given cultivar, that a dough weakening effect is observed as evident by a decrease in resistance to extension and an increase in extensibility. In this case, water would be having a larger plasticizing effect.

In the literature, damaged starch is known to have higher water absorption capabilities than native starch, and to compete with gluten proteins for water within the dough (Goesaert et

al., 2005; Mak, 2009; Barak et al., 2013). This competition can lead to water mobility within the dough, which can impact both the structure of the gluten network and the handling properties of the dough. In the solvent retention capacity tests, results indicated that the higher amounts of damaged starch within McKenzie and Harvest led to a lower gluten performance index than that of Pembina and Roblin. Within the current study it is postulated that the level of damaged starch played a role in the gluten network formation and strength given that it competes with the gluten proteins for hydration. It was concluded that a greater amount of damaged starch can have a detrimental impact on the gluten network. This was observed in dough prepared with McKenzie and Harvest which had higher levels of damaged starch and lower quality gluten than that of Pembina. As such, McKenzie and Harvest were unable to overcome the weakening impact of the higher level of damaged starch.

In conclusion of study 1, having high protein content is important and inter-related to dough handling properties and strength, however it's not the driving factor. This was displayed with Pembina having the lowest protein content of the four cultivars but showing greater strength than the higher protein cultivars of Roblin, McKenzie and Harvest. The higher gluten quality (in regard to the higher gluten index, gluten performance index and glutenin: gliadin ratio) and lower damaged starch levels of Pembina/Roblin cultivars allowed for the formation of stronger gluten networks and thus formed stronger dough than McKenzie and Harvest. Reasons why Pembina was stronger than Roblin are less clear. However, this may be attributed to compositional differences within the HMW-GS fractions; and/or the higher levels of LMW-GS proteins in Pembina that may act as chain extenders, cross-linkers, or branch points all of which create a buildup of the gluten network and enhance interactions within the gluten network polymers. Aside from gluten protein quality accounting for the differences between the cultivars strength, damaged starch and water addition (below or above the FAB needed for optimal dough development) would also have an impact on the dough strength. Since all cultivars were mixed with optimal water additions to their optimal dough development, differing water additions were not considered to have a significant impact relative to the strength and dough handling properties.

#### **6.4 Impact of NaCl on dough handling, stickiness and water mobility on doughs prepared from different CWRs wheat cultivars**

It is well documented that NaCl is necessary for enhancing the viscoelastic properties of dough by increasing the level of gluten protein interactions via charge shielding on the proteins (Miller & Hoseney, 2008; Uthayakumaran et al., 2011; Belz et al., 2012). However, within the literature there have been contradictory findings among researchers as to the effect of salt reduction on empirical vs fundamental dough rheology and stickiness (Larsson, 2002; Lynch et al., 2009; Beck et al., 2012a; McCann & Day, 2013; Tuhumury et al., 2014). Because of the lack of clarity, the purpose of the second study was to gain a better understanding of dough rheology (small strain → large deformation rheology), dough stickiness and water mobility within dough prepared from different cultivars over a range of NaCl levels (0-4%), and their inter-relationship with the gluten network. It was hypothesized that with higher NaCl levels there would be an increase in protein-protein interactions as the result of increased charge shielding leading to a more organized gluten network and a more viscoelastic non-sticky dough. Whereas, at lower NaCl levels it was hypothesized that dough stickiness would increase as the result of decreased protein-protein interactions resulting in an unorganized multidirectional gluten network which displayed increased viscous properties and decreased dough strength.

##### *a) Comparison of empirical and fundamental rheological data for dough prepared at the 2% NaCl level for different wheat cultivars*

Rheological analysis obtained from large deformation (e.g., farinograph, mixograph, extensigraph and creep recovery) often show different responses than when small oscillatory shear rheometry is used, an observation that is more often seen when materials contain HMW polymers (Dobraszczyk & Morgenstern, 2003). Differences observed between the rheological techniques of dough are thought to be caused by: a) insensitivities within the linear viscoelastic regime of the fundamental rheology, to be able to differentiate responses of gluten polymers having a wide molecular weight distribution (MW) (Dobraszczyk & Morgestern, 2003); b) differences in water content, where its addition above or below the optimal FAB amount can result in softening or stiffening of the dough, respectively; c) the starch content, where starch-starch interactions have been hypothesized to overshadow rheological responses of the gluten polymers at low strains (McCann & Day, 2013; Meerts et al., 2017). This has been seen in



multiple studies examining different cultivars with varying strengths where the weaker flour outperforms the stronger flour in the oscillatory shear, however when the larger strains of creep recovery or uniaxial/biaxial extension are used the stronger flour performs better than the weaker (Dobraszczyk & Salmonowicz, 2008; McCann & Day, 2013; Tuhumury et al., 2014; Meerts et al., 2017). In Table 6.2, data from empirical (e.g., resistance to extension) and fundamental (e.g.,  $\tan \delta$  - oscillatory; and  $J_{\max}$  - creep recovery) rheology measurements at the 2% NaCl level were contrasted using data reported from study 1 and 2 where dough prepared with each cultivar was mixed with the optimal FAB. Similar trends in the data were found for all four cultivars using all measurement techniques when mixed with the optimal FAB, where Pembina was found to form the strongest dough, followed by Roblin and McKenzie, and then Harvest (Table 6.2). Findings suggest that both empirical and fundamental techniques provide complementary information, where water addition or starch-starch interactions (as described above) did not seem to overshadow the sensitivities of the small and large strain measurements ( $\tan \delta$  and  $J_{\max}$  respectively).

*b) Effects of varying levels of NaCl on dough prepared by the different wheat cultivars on dough strength and stickiness*

As discussed earlier, the strength of the gluten network is impacted by the protein content and composition, pentosans, starch-starch interactions and water addition. Because of this, depending on the cultivar (i.e., flour composition and quality) and its preparation (i.e., water addition and mixing) contradictory findings have been reported in studies examining NaCl levels and dough rheology using small strain oscillatory deformation. For instance, some studies have reported that increasing the levels of NaCl resulted in an increase in  $G'$  (Larsson, 2002; Beck et al., 2012a) and a decrease in  $\tan \delta$  (Beck et al., 2012a) both of which indicates an increase in the gluten polymer network's strength and ability to store energy. Whereas others have found that increasing NaCl appeared to cause a decrease in the  $G'$  and either no change or an increase in  $\tan \delta$ , both of which indicates a decrease in the elastic characteristics of the gluten network (Lynch et al., 2009; McCann & Day, 2013; Tuhumury et al., 2014). However overwhelmingly within the literature studies, it is known that NaCl has a strengthening effect on doughs prepared from wheat flours (Larsson, 2002; Uthayakumaran, 2011; Beck et al., 2012a; Belz et al., 2012). In contrast; Lynch et al. (2009), McCann and Day (2013) and Tuhumury et al. (2014) also reported

Table 6.2. A comparison between empirical and fundamental rheological measurements on dough prepared using different cultivars at the 2% NaCl level. Data represent the mean  $\pm$  one standard deviation.

Cultivar	<sup>1</sup> Resistance to extension (N)	<sup>2</sup> $\tan \delta$	<sup>2</sup> Maximum creep compliance ( $J_{\max}$ ) (1/mPa)
Pembina	$0.31 \pm 0.02$	$0.361 \pm 0.004$	$1.84 \pm 0.13$
Roblin	$0.25 \pm 0.01$	$0.386 \pm 0.004$	$3.06 \pm 0.59$
McKenzie	$0.23 \pm 0.00$	$0.387 \pm 0.002$	$3.07 \pm 0.18$
Harvest	$0.18 \pm 0.01$	$0.383 \pm 0.003$	$4.99 \pm 0.55$

Notes: (1) Data taken from study 1; (2) Data taken from study 2.

a strengthening trend with increasing NaCl levels when using large deformation testing (uniaxial extension). The weakening of dough in response to added NaCl in some studies is most likely associated with increased sensitivity of the small strain testing to changes in water mobility and increased starch-starch interactions. Within the present study it is believed that the migration of water, with the addition of salt, from free water to the starch-fraction contributes to the contradictory findings in oscillatory shear examining the effect of sodium chloride. For instance, in the present study the  $\tan \delta$  value indicates that the weakest cultivar of Harvest has a similar  $\tan \delta$  (elastic behaviour) as the stronger dough producing cultivar of Roblin. However, given the creep recovery and extensigraph findings Roblin is stronger than Harvest. In contrast, Pembina does not have the same significant migration of water to the starch and therefore showed similar strength trends in the oscillatory shear, creep recovery and extensigraph.

In the present study, all cultivars showed a strengthening effect with increasing levels of NaCl as measured by oscillatory shear and creep recovery rheology, as evident by lower  $\tan \delta$  and  $J_{\max}$  values at higher NaCl levels (Table 6.3). Stickiness values were also found to increase with the reduction in NaCl for all cultivars examined, however Pembina was least impacted by NaCl level and did not change between the 1 and 2% NaCl level (Table 6.3). It was believed that the greater number of gluten-gluten interactions between the polymers within Pembina led to

Table 6.3. Effect of NaCl level on fundamental rheological parameters, stickiness values and % water associated with the starch-fraction for doughs prepared from different cultivars. Data represent the mean  $\pm$  one standard deviation.

<b>Cultivar</b>	<b>NaCl (%)</b>	<b><math>\tan \delta</math></b>	<b><math>J_{\max}</math> (1/mPa)</b>	<b>Stickiness (N)</b>	<b>Water associated with the starch-fraction (%)</b>
Pembina	0	$0.397 \pm 0.001$	$3.47 \pm 0.13$	$0.55 \pm 0.03$	$19.3 \pm 2.17$
	1	$0.368 \pm 0.006$	$2.42 \pm 0.47$	$0.47 \pm 0.03$	-
	2	$0.361 \pm 0.004$	$1.84 \pm 0.13$	$0.47 \pm 0.01$	$21.6 \pm 1.07$
	4	$0.354 \pm 0.004$	$1.24 \pm 0.05$	$0.51 \pm 0.01$	-
Roblin	0	$0.422 \pm 0.008$	$5.38 \pm 0.99$	$0.56 \pm 0.02$	$16.9 \pm 1.04$
	1	$0.393 \pm 0.004$	$3.71 \pm 0.32$	$0.52 \pm 0.02$	-
	2	$0.386 \pm 0.004$	$3.06 \pm 0.59$	$0.55 \pm 0.01$	$25.3 \pm 1.20$
	4	$0.378 \pm 0.001$	$1.98 \pm 0.11$	$0.62 \pm 0.00$	-
McKenzie	0	$0.434 \pm 0.005$	$7.25 \pm 1.45$	$0.65 \pm 0.01$	$18.4 \pm 0.33$
	1	$0.397 \pm 0.001$	$4.36 \pm 0.18$	$0.61 \pm 0.02$	-
	2	$0.387 \pm 0.002$	$3.07 \pm 0.18$	$0.64 \pm 0.02$	$22.7 \pm 0.21$
	4	$0.360 \pm 0.005$	$1.35 \pm 0.31$	$0.59 \pm 0.00$	
Harvest	0	$0.431 \pm 0.003$	$11.08 \pm 0.91$	$0.69 \pm 0.02$	$15.2 \pm 1.24$
	1	$0.397 \pm 0.003$	$7.01 \pm 0.91$	$0.62 \pm 0.02$	-
	2	$0.383 \pm 0.003$	$4.99 \pm 0.55$	$0.57 \pm 0.01$	$20.3 \pm 1.14$
	4	$0.371 \pm 0.006$	$2.77 \pm 0.47$	$0.65 \pm 0.02$	-

Note: hyphen (-) indicates not measured.

a more structured dough where water was adsorbed to the glutens surface and also trapped within pores within the gluten network.

It is postulated that the differences seen within the dough rheology and stickiness data as a function of NaCl level reflects differences in protein composition between cultivars, particularly the HMW and LMW-GS fractions. Butow et al. (2002) found that different sensitivities to NaCl addition among cultivars could be explained by grouping flour cultivars based on their HMW-GS make-up. The authors also showed that cultivars that had an over

expression of the HMW-GS of Bx7 resulted in significantly greater increases in dough strength parameters with the addition of salt than cultivars without the over expressed gene. Indicating that less salt may be necessary to provide strength to the dough within the HMW-GS Bx7 over expression cultivars. In the first study of this research Pembina, for instance, had a higher glutenin: gliadin ratio than Harvest but similar to that of Roblin, and had a lower HMW: LMW-GS ratio than Roblin. Pembina also had a similar HMW-GS amount to that of the weaker cultivar Harvest thus pointing to the role of HMW-GS composition and the combined role of LMW-GS in the reduced sodium environments in maintaining dough strength and decreased dough stickiness. With regard to HMW-GS composition it has been indicated that within the repeat regions, of the HMW-GS, certain amino acid arrangements result in loops and others in trains, therefore some subunits will create more elasticity or resistance within the dough (Belton, 2012). Experiments presented in Belton (2012) also indicated that greater resistance to extension resulted from perfect repeat regions that lead to higher levels of beta sheets. The role of LMW-GS in Pembina are proposed to act as chain extenders (lengthening the glutenin polymers), cross-linkers (interconnecting the aligned HMW-GS polymers) and chain terminators (with the potential to create physical entanglements between the glutenin polymer proteins upon deformation of the dough). The role of NaCl in dough is to shield the charges on the gluten polymers to promote closer gluten-gluten interactions facilitated by hydrophobic interactions. As NaCl is decreased, the numbers of these interactions also decrease and leads to a weaker less interconnected network. Differences in composition of the HMW-GS proteins is thought to lead to differences in available charges to be shielded by the NaCl leading to some NaCl sensitivities in some cultivars and not others. This was reflected in the rheological data associated with a reduction in strength with decreasing levels of NaCl, and differences in strength among the different cultivars (Table 6.3).

In the present study, Pembina formed a strong dough capable of storing elastic energy within the gluten network as evident by oscillatory and creep recovery rheology (e.g., highest  $G'$ , lowest  $\tan \delta$ , lowest  $J_{\max}$ , highest  $J_{el}$  and greatest resistance to extension) and also formed a non-sticky dough. In contrast, Harvest formed a weaker dough, less capable of storing/ transferring energy throughout the gluten network, that was sticky. Relationships between dough stickiness and dough rheology have been reported by Dobraszczyk (1997). The level of ordering within the gluten network and charge shielding occurring is postulated to impact stickiness, where a less

ordered (and weaker) gluten network would have more unbound water (weakly absorbed free water that is more mobile near the surface) and less water strongly absorbed entrapped within the gluten network resulting in stickier dough. Based on the correlations found by Dobraszczyk (1997) between dough rheology and stickiness and the findings in the current study, dough rheology may be a good indicator of whether a sticky dough is formed, although the use of fundamental rheology as the only predictor may be pre-mature, as other factors (e.g., water mobility and distribution) complicate the picture.

*c) Effects of varying levels of NaCl on water mobility within dough prepared by the different wheat cultivars and the impact on dough strength and stickiness*

The migration of water in dough prepared from different cultivars, with differing NaCl levels, has an impact on dough strength. Based on the TGA curve fitting, it is hypothesized that the gluten network is not the only factor determining the presence of dough stickiness in reduced sodium environments. Rather, there is also the impact of the relationship between the free water (from the diffuse layer near the dough's surface that is weakly absorbed, more mobile) and water associated with the starch-fraction (as either unbound or bound water). For instance, at the 0% NaCl level the percentage of water associated with the starch-fraction was less than at the 2% NaCl level for Roblin, McKenzie and Harvest (Table 6.3). This trend was also observed for Pembina, however the change was not significant. This movement was also associated with decrease in dough stickiness (Table 6.3). Beck et al. (2012a) suggested that the unbound water within dough might be responsible for dough stickiness. In other works, it has been shown that doughs' excess in water resulted in increased stickiness (Dhaliwal et al., 1990; Chen & Hosney, 1995a; Jekle & Becker, 2011). Larsson (2002) also showed a change in the water's association with the starch-fraction with the addition of NaCl as determined by ultracentrifugation of the dough. These findings along with the findings in the present study indicate an interaction of both the gluten network formation and strength and the migration of water in the presence of dough stickiness with changes in NaCl levels.

*d) Impact of a constant water addition on rheological properties and stickiness of dough prepared with different wheat cultivars at the 2% NaCl level*

All doughs prepared in this research were mixed using the optimal FAB water content in order to ensure each dough was mixed to its optimal development. However in doing so, each dough contained different levels of water. For comparative purposes, rheological data (e.g., resistance to extension,  $\tan \delta$  and  $J_{\max}$ ) and stickiness values were all normalized to the same FAB water content as Pembina. The normalization procedure was not applied to data as a function of NaCl content (for each cultivar), since differences were considered minor relative to differences between cultivars. Normalization of the data revealed differences between the rheological techniques in terms of trends observed with dough strength, however by normalizing the water addition to that of Pembina this meant a decrease in water for the other flour cultivars and it can be seen in Table 6.4 that this results in both an increase in the dough strength parameters (or a stiffening of the dough) and a decrease in dough stickiness. For instance, resistance to extension (empirical rheology) data found Pembina to offer the greatest resistance (i.e., stronger dough), followed by Roblin and McKenzie, and then Harvest, which was the same trend as the normalized data. However, the different cultivars did notice an increase in resistance to extension which was expected given that when water is decreased in a dough below the FAB value the dough gets stiffer. Normalization of the  $\tan \delta$  (fundamental rheology) data in Table 6.4 exhibited a change in the trend from that of the raw data (given in Table 6.2). Pembina no longer exhibited the greatest elastic behaviour where Roblin and Harvest (which have the highest water additions) were found to have an increase in elasticity similar to that of Pembina by decreasing the amount of water. Other researchers have also found that with less water there was an decrease in  $\tan \delta$  (Khatkar et al., 1995; Jekle & Becker, 2011), however others found that there was no change in  $\tan \delta$  with a decrease or increase in water as the  $G'$  and  $G''$  changed in the same order of magnitude (Navickis et al., 1982; Berland & Launay, 1995; Letang et al., 1999). From this, it is noteworthy to mention that the normalization of the data in the present study is a very simplistic look at the impact of water addition on dough rheology and strength/stickiness. Water addition is not the sole determinant of dough rheology, and other factors such as dough formulation (flour composition and quality, salt level, etc.) and mixing conditions impact dough rheology as well. For the maximum creep compliance (fundamental rheology) the trend stayed the same with Pembina experiencing the least deformation followed by Roblin and McKenzie

Table 6.4. A comparison between empirical and fundamental rheology measurements, and stickiness on dough prepared using different cultivars at the 2% NaCl level. Data represent the mean  $\pm$  one standard deviation. All data is normalized to the FAB of Pembina.

Cultivar	FAB (% to 14% w.b) (Studies 1/2)	<sup>1</sup> Resistance to extension (N)	<sup>2</sup> tan $\delta$	<sup>2</sup> Maximum creep compliance (J <sub>max</sub> ) (1/mPa)	<sup>2</sup> Stickiness (N)
Pembina	61.5/61.9	0.31 $\pm$ 0.02	0.361 $\pm$ 0.004	1.84 $\pm$ 0.13	0.47 $\pm$ 0.01
Roblin	65.3/65.0	0.27 $\pm$ 0.01	0.368 $\pm$ 0.004	2.91 $\pm$ 0.56	0.52 $\pm$ 0.01
McKenzie	63.3/64.1	0.23 $\pm$ 0.00	0.374 $\pm$ 0.002	2.97 $\pm$ 0.17	0.62 $\pm$ 0.01
Harvest	64.9/65.5	0.19 $\pm$ 0.01	0.362 $\pm$ 0.003	4.72 $\pm$ 0.52	0.54 $\pm$ 0.01

Notes: (1) Data taken from study 1; (2) Data taken from study 2. FABS values were slightly different between studies 1 and 2, since the research studies 2 and 3 were completed first, followed by the compositional analysis (in which the FAB were repeated).

which were similar, and then Harvest which experienced the highest amount of deformation. Wang and Sun (2002) found that doughs at both their optimal and a fixed water level did not notice major changes to their creep recovery parameters. Findings suggest that small strain fundamental rheology (tan  $\delta$ ) is more sensitive to water addition than the larger fundamental rheology (J<sub>max</sub>) and empirical techniques. In all cases, normalization to Pembina would result in the amounts of water added being below each cultivars' FAB optimal value, which would cause a stiffening of the dough network and give rise to higher resistance to extension. In the case of dough stickiness all flour cultivars exhibit a decrease in stickiness with normalization of water levels to that of Pembina. However, the trend still remained the same with Pembina still showing the lowest dough stickiness. The decrease in dough stickiness with a reduction in the water added to the dough was expected with normalization of the data due to the findings of Chen and Hosney (1995a) and Jekle and Becker (2011) where doughs mixed with water addition both above and below the FAB resulted in an increase and decrease in stickiness respectively.

In summary, based on study 1 and 2 together, it is concluded that dough stickiness in reduced sodium formulations is governed by a combination of the gluten network formation as dictated by protein quality (i.e., glutenin: gliadin ratio, HMW-GS: LMW-GS ratio and greater presence of high quality LMW-GS), the level of damaged starch, and the changes in water mobility. It is postulated that the formation of a strong gluten network made up of high quality HMW-GS and LMW-GS is capable of mitigating the effects of dough stickiness in reduced sodium environments. However, in weaker gluten networks with higher damaged starch the occurrence of dough stickiness becomes more apparent through the change in water's mobility between water associated with the starch-fraction and that of free water (which is not entrapped within the gluten network). Therefore, it is concluded that cultivar selection is of the utmost importance when preparing low sodium dough formulations in the prevention of a sticky dough.

### **6.5 Impact of salts from the lyotropic series on dough handling, stickiness and water mobility in doughs prepared from different CWRS wheat cultivars**

The use of alternative salts from the lyotropic series has been investigated by a number of researchers as a means to achieving NaCl reduction while still maintaining dough handling properties and final bread quality (Salovaara, 1982ab; Kinsella & Hale, 1984; Preston, 1989; He et al., 1992; Butow et al., 2002; Charlton et al., 2007; Braschi et al., 2009; Kaur et al., 2011; Uthayakumaran, 2011; Tuhumury et al., 2016ab). Within these studies the replacement of either the cation ( $\text{Na}^+$ ) or anion ( $\text{Cl}^-$ ) or both have been examined, however within the current study only the replacement of the cation was studied. Within aforementioned studies, there was no examination of the effect on dough stickiness which is a major problem within reduced NaCl formulations. Therefore, the purpose of the final part of the research was to gain a greater understanding of the influence that the replacement of NaCl with salts from the lyotropic series ( $\text{NH}_4\text{Cl}$ ,  $\text{KCl}$ ,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ , and  $\text{MgSO}_4$ ) would have on the rheology, stickiness, morphology and water mobility within dough prepared from a strong/non-sticky (Pembina) and weak/sticky (Harvest) dough producing CWRS flour at both the 1% level and 2% level. It was hypothesized that those salts with cations that are non-chaotropic ( $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ) in nature would result in a strengthening of the gluten network and therefore a non-sticky dough with decreased water mobility because non-chaotropic cations tend to induce a greater amount of protein-protein interactions. On the other hand, the chaotropic cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) are hypothesized to result in



a weakening effect on the gluten network and therefore an increase in dough stickiness and an increase in water mobility because the chaotropic ions result in greater protein hydration.

*a) The impact of concentration of salts from the lyotropic series on the dough handling properties of dough prepared from Pembina (strong/non-sticky) and Harvest (weak/sticky) wheat cultivars*

Overall, for doughs produced with both flour cultivars using salts from the lyotropic series, an increase in strength was observed at the 2% level relative to the 1% level, for each given salt-type. However, a greater distinction between the dough strengthening effects of the more non-chaotropic cations as compared to the weakening effects of the more chaotropic cations was observed at the 2% level vs the 1% level. It has been reported that higher concentrations, above ~ 0.3-0.5 M, for both the cations and anions of salts from the lyotropic series exhibit a greater influence on the level of protein aggregation in conformity to their ranking in the series, with chaotropic effects becoming more pronounced (Kinsella & Hale, 1984; Preston, 1989; He et al., 1992; Melnyk et al., 2011; Tuhumury et al., 2016b). In contrast, at lower salt concentrations ~0.1-0.3 M a similar effect is seen on protein aggregation due to the similarities in electrostatic charge shielding of all ions. It is presumed that the more ionic environment would offer greater charge shielding to the glutenin and gliadin proteins present, enabling a greater amount of hydrophobic protein-protein interactions (and ordering within the gluten network) to occur (Preston, 1989; Melnyk et al., 2011).

In combination to the effect of the salt-type concentration, there is also the effect of ion charge as well. The present research focuses on removing the cation ( $\text{Na}^+$ ) as part of an overall sodium reduction approach. However, in the literature, it has been shown that replacing NaCl with anions from the lyotropic series has a much greater influence on dough handling due to greater ability of the anion to induce conformational changes on the gluten proteins (Kunz, 2010; Melnyk et al., 2011; Tuhumury et al., 2016a,b). In the mentioned studies, it has been indicated that anions are more effective at shielding the charge on the gluten proteins to induce greater protein-protein interactions than cations do. As such, our approach to use cations may also have lead to reduced differences between the salt-types given that the anion chloride (which would provide a greater influence over the cations) remains constant.

In addition, greater dough strength was exhibited for doughs produced using salts from the lyotropic series with Pembina relative to Harvest, regardless of the salt-type. This finding was proposed to stem back to differences in protein composition/quality (higher glutenin: gliadin of Pembina relative to Harvest) (study 1). This would lead to increased dough strength and reduced stickiness because the flour has a greater amount of HMW glutenin polymers that interact more strongly via hydrophobic interactions with less gliadins disrupting the formation of a more ordered gluten network. It was also suggested by Melnyk et al. (2011) that the effect of the salts on the gluten protein aggregation within the dough may be influenced by differences in flour composition (i.e., protein content and composition/quality). The cultivar dependence for the effect of salt-type observed is also thought to stem from the higher quality HMW-GS and LMW-GS of Pembina relative to Harvest (given that they have equal amounts of HMW-GS); He et al. (1992) also reported that higher quality flour (i.e., higher glutenin: gliadin ratio) outperformed the weaker cultivar with the differing salts from the lyotropic series. The rationale for these findings are that the higher quality glutenin in the stronger dough producing flour have greater surface hydrophobicity than those of the weaker quality gluten (He et al., 1992). Their conclusion was based off of the findings of Chung and Pomeranz (1979) who found that the glutenin from the poor flour was less hydrophobic than that of the good flour through the use of hydrophobic gel separation. From this it is hypothesized that there is similarly a higher surface hydrophobicity of Pembina HMW-GS (high quality) than Harvest HMW-GS (lower quality) which would help account for why higher salt levels are necessary to strengthen Harvest and promote good dough handling properties. He et al. (1992) also reported that more salt was needed to maintain good proof height and loaf volume if the flour used was of poor quality.

*b) The impact of salt-type from the lyotropic series at the 1% salt levels on the dough handling properties of dough prepared from Pembina and Harvest*

Based on the overall goal of the research a more detailed examination on the dough handling properties at the 1% salt level will be given. In general, when examining the replacement of cations in NaCl and the impact on fundamental rheological properties of Pembina and Harvest (Table 6.5) it can be seen that the overall trends tend to follow the lyotropic series in regard to providing a strengthening (non-chaotropic) or weakening (chaotropic) effect relative to NaCl. However, these differences relative to NaCl tended not to be significant with the use of

Table 6.5 Fundamental rheological properties and stickiness of doughs prepared with salts from the lyotropic series at the 1% level.

Flour Cultivar	Salt (1% on flour weight basis)	Salt (mol/L)	Tan $\delta$	J <sub>el</sub>	Stickiness (N)
Pembina	NH <sub>4</sub> Cl	0.30	0.353 $\pm$ 0.002	0.62 $\pm$ 0.01	0.31 $\pm$ 0.02
	KCl	0.22	0.372 $\pm$ 0.006	0.51 $\pm$ 0.04	0.32 $\pm$ 0.02
	NaCl	0.28	0.368 $\pm$ 0.006	0.57 $\pm$ 0.05	0.47 $\pm$ 0.03
	CaCl <sub>2</sub>	0.15	0.367 $\pm$ 0.006	0.54 $\pm$ 0.03	0.36 $\pm$ 0.01
	MgCl <sub>2</sub>	0.17	0.372 $\pm$ 0.003	0.51 $\pm$ 0.02	0.44 $\pm$ 0.02
	MgSO <sub>4</sub>	0.13	0.362 $\pm$ 0.004	0.61 $\pm$ 0.01	0.34 $\pm$ 0.03
Harvest	NH <sub>4</sub> Cl	0.29	0.390 $\pm$ 0.006	0.33 $\pm$ 0.02	0.39 $\pm$ 0.01
	KCl	0.20	0.405 $\pm$ 0.004	0.22 $\pm$ 0.02	0.63 $\pm$ 0.01
	NaCl	0.26	0.397 $\pm$ 0.003	0.29 $\pm$ 0.03	0.62 $\pm$ 0.02
	CaCl <sub>2</sub>	0.14	0.397 $\pm$ 0.009	0.26 $\pm$ 0.06	0.52 $\pm$ 0.03
	MgCl <sub>2</sub>	0.16	0.413 $\pm$ 0.005	0.19 $\pm$ 0.02	0.60 $\pm$ 0.02
	MgSO <sub>4</sub>	0.13	0.388 $\pm$ 0.004	0.35 $\pm$ 0.04	0.60 $\pm$ 0.02

oscillatory shear, but became slightly more apparent with the use of the larger deformation of creep recovery (although still no major differences). When examining the results from the creep recovery (Table 6.5), doughs prepared with Harvest in the presence of KCl and MgCl<sub>2</sub> gave lower J<sub>el</sub> values indicating a weakening of the gluten network, whereas all others were similar in magnitude with the exception of MgSO<sub>4</sub> which showed higher J<sub>el</sub> values than NaCl. This similar strengthening effect of MgSO<sub>4</sub> on dough strength/stability was found by Salovaara (1982a) and Kaur and others (2011). They postulated that the stabilizing effect of the sulfate (non-chaotropic anion) relative to the chloride anion outweighed the destabilizing effect of the magnesium cation (chaotropic). For doughs prepared with Pembina only those prepared with NH<sub>4</sub>Cl gave higher J<sub>el</sub> values indicating increased dough elasticity. In contrast, KCl and MgCl<sub>2</sub> showed reduced J<sub>el</sub> values relative to NaCl suggesting that those salts caused a decrease in network recovery. Uthayakumaran (2011) also found increased dough strength with the use of NH<sub>4</sub>Cl. Tuhumury and others (2016a) reported for gluten washed dough samples that the non-chaotropic cations

resulted in similar but higher  $G'$  and  $G''$  values than those of the chaotropic cations. However, the authors did not find differences between the strong and weak cultivars they used with each salt type. The lack of major significant differences within the present study and that by Tuhumury et al. (2016a) is most likely due to the insensitivities of the small strain oscillatory shear to detect the small changes within the ordering of the gluten network based off differences in MW distributions, water mobility and starch-starch interactions in response to the different salt types. Thus, when examining the impact of salts from the lyotropic series within a given flour cultivar on the dough handling properties, the larger strain of creep recovery or extensional rheology may be more beneficial. It is also interesting to note that other studies have found that KCl often provides similar effects on dough handling properties as NaCl (Salovaara, 1982a; Kaur et al., 2011; Tuhumury et al., 2016a) given that they are ranked close/equivalent in the lyotropic series; however in the present study this similar effect is not consistently seen, which might be explained by the differences in molarity (Table 6.5). Within the present study, addition of salt was done on a flour weight basis not on an equivalent molarity basis to NaCl. It can be seen that the cations of larger size have lower concentrations than the smaller cations when added at the 1% level of salt on a flour weight basis. If the same molarity was used, as in Butow et al. (2002), then greater differences may have been seen between the different cations and KCl may have performed similar to NaCl.

With regards to the impact of the alternative salts on dough stickiness, dough prepared with Pembina showed lower dough stickiness than Harvest in all cases. For doughs prepared with both Pembina and Harvest, stickiness was found to show the greatest reduction with the use of  $\text{NH}_4\text{Cl}$ . There have been no studies, to our knowledge investigating the effect of alternative salts on dough stickiness, however it was concluded from the current results that  $\text{NH}_4\text{Cl}$  decreases dough stickiness for both flour cultivars due to the increase in dough handling properties, dough strength and microstructure formation/organization. However, dough strength and stickiness did not seem to be as strongly associated in this study involving lyotropic series, as compared to the second study with only NaCl at different levels. It was hypothesized that chaotropic cationic salts from the lyotropic series would cause a decrease in strength which would then translate into an increase in stickiness and vice versa with the use of non-chaotropic cationic salts. However, all alternative salts resulted in either a decrease or no change in dough stickiness relative to NaCl despite changes in dough strength parameters. For instance, generally

when comparing the  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  relative to  $\text{NaCl}$ ,  $\text{NH}_4\text{Cl}$  resulted in a strengthening effect whereas  $\text{MgCl}_2$  resulted in a weakening effect. However,  $\text{NH}_4\text{Cl}$  significantly reduced stickiness in both Pembina and Harvest, but instead of increasing stickiness  $\text{MgCl}_2$  resulted in a decrease in both. It is also interesting to note that only  $\text{NH}_4\text{Cl}$  reduced dough stickiness in Harvest to values closer to that of Pembina.

When examining the effect of the  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  (only) on water mobility (i.e., unbound [freezable] vs bound [un-freezable]) it was found that Pembina showed an increase in the amount of freezable water relative to  $\text{NaCl}$  (Table 6.6). This is believed to occur because of different cation-water interactions occurring related to differences in different cation sizes, their hydration radii charge density, and molarity (Butow et al., 2002). For Pembina, alternative salts resulted in an increase in water associated with gluten relative to the  $\text{NaCl}$  which may be the reason why both salt-types decreased stickiness relative to  $\text{NaCl}$  at the 1% level. In the case of Harvest,  $\text{NH}_4\text{Cl}$  had similar FWC to  $\text{NaCl}$ , whereas the presence of  $\text{MgCl}_2$  increased it (Table 6.6). However,  $\text{NH}_4\text{Cl}$  decreased stickiness and  $\text{MgCl}_2$  resulted in similar stickiness to  $\text{NaCl}$ . When looking at association of water it can be seen that  $\text{MgCl}_2$  resulted in similar starch water association as  $\text{NaCl}$  whereas  $\text{NH}_4\text{Cl}$  resulted in less water associated with the starch. Based on these findings, stickiness seems to be more related to changes in water distribution within the dough, along with the strength of the gluten network.

In conclusion of study 3,  $\text{NH}_4\text{Cl}$  is the most effective at enhancing dough strength and decreasing dough stickiness in the reduced salt formulations of salts from the lyotropic series in both the strong dough producing cultivar Pembina and the weaker dough producing cultivar Harvest. In the presence of both the non-chaotropic and chaotropic salts of the lyotropic series, the higher quality gluten in Pembina is postulated to result in greater strength and reduced stickiness as compared to Harvest. The higher quality also helps account for the decreased sensitivity of Pembina to the chaotropic effects of the salts as compared to Harvest. The combined analysis of the effect of salts from the lyotropic series on dough strength/rheology, stickiness, and water mobility in two cultivars showed that the dough stickiness phenomenon is an increasingly complicated issue as all alternative salts resulted in decreased stickiness, however had differing effects on water association and dough strength which was cultivar dependant. In the future, investigation of replacement with cations and anions from the lyotropic series on a % flour weight basis and an equimolar basis should be studied to better understand

Table 6.6 Water mobility and dough stickiness within dough prepared from Pembina and Harvest with salts from the lyotropic series at 1% (NH<sub>4</sub>Cl, NaCl, MgCl<sub>2</sub>).

Flour Cultivar	Salt (1%)	Freezable water content (g ice/ g dough d.b)	Stickiness (N)	Free water (%)	Water Associated with the starch- fraction (%)	Water associated with the gluten- fraction (%)
Pembina	NH <sub>4</sub> Cl	0.43 ± 0.00	0.31 ± 0.02	17.6 ± 0.95	19.1 ± 1.06	63.3 ± 2.01
	NaCl	0.39 ± 0.01	0.47 ± 0.03	20.6 ± 0.19	22.1 ± 1.79	57.3 ± 1.98
	MgCl <sub>2</sub>	0.44 ± 0.01	0.44 ± 0.02	15.3 ± 0.45	21.2 ± 1.02	63.5 ± 0.57
Harvest	NH <sub>4</sub> Cl	0.45 ± 0.01	0.39 ± 0.01	19.4 ± 1.69	18.7 ± 1.19	61.9 ± 0.50
	NaCl	0.46 ± 0.01	0.62 ± 0.02	13.9 ± 0.71	23.8 ± 1.56	62.4 ± 2.27
	MgCl <sub>2</sub>	0.48 ± 0.01	0.60 ± 0.02	16.3 ± 1.96	24.1 ± 1.36	59.5 ± 0.59

the inter-relationship between protein composition from different cultivars, water mobility, dough strength and dough stickiness.

## 6.6 Summary

In summary, research arising from this thesis indicates that careful cultivar selection of those with high quality flour composition (high glutenin: gliadin ratio, combined with a higher amount of LMW-GS, and lower levels of damaged starch) combined with the use of NH<sub>4</sub>Cl would make for a promising strategy for producing dough with good handling properties within a low sodium environment.

## 7. GENERAL CONCLUSIONS

The overall aim of this research was to investigate the underlying mechanisms leading to the sticky dough phenomenon within reduced sodium dough formulations by focusing on gluten network strength and formation as it relates to dough handling properties. To achieve this, four different Canada Western Red Spring (CWRS) wheat flour cultivars known to have good, average and poor dough handling properties in low sodium environments were examined. First the compositional characteristics were analyzed and related to dough strength in a standard NaCl formulation. Dough rheology, dough morphology, dough stickiness and water mobility were utilized as indicators of formulation changes with a range of NaCl levels and alternative salts from the lyotropic series.

The first stage of this research (Chapter 3) analyzed the effects of compositional characteristics of four different CWRS wheat cultivars on dough formation and strength. Overall the flour cultivar Pembina proved to form doughs with the best handling properties with respect to having the highest ratio of resistance to extension to extensibility, which gives an indication of a gluten network's elasticity. Pembina also appears to form more directionally oriented gluten network in the CLSM micrographs, having the highest particle count indicating the formation of an organized and continuous gluten network. On the other hand, the flour cultivar Harvest had the worst dough handling properties with the lowest resistance to extension indicating a weaker gluten network. Harvest also had the most porous and multidirectional oriented gluten network with the lowest particle count (greatest amount of gluten protein aggregation) as indicated by the CLSM micrographs and image analysis. The differences in the dough strength and gluten network formation/structure are postulated to be attributed to the gluten quality index, ratio of glutenin: gliadin proteins, and differing levels of damaged starch. More specifically, differences in dough properties between the cultivars was attributed to differences in subunit composition within the HMW insoluble glutenin subunit fraction, and also differences in the levels of LMW glutenin subunit and gliadin fractions. Findings from this study indicate that gluten network formation and strength is dependent upon gluten quality over quantity given Pembina has the

lowest protein content of all the cultivars and forms the strongest gluten network. Findings from this study give rise to the hypothesis that the insoluble LMW glutenin subunits play a greater role, than originally hypothesized, in maintaining the gluten network formation and strength in reduced sodium environments.

The second stage of this research (Chapter 4) examined the effect of NaCl (0-4%) levels on the handling properties of doughs prepared from different CWRS wheat cultivars. Overall, rheological results indicated that the strength of the gluten network increased with increasing levels of NaCl; however the magnitude was cultivar dependent. The two stronger cultivars of Pembina and Roblin had the lowest dough stickiness relative to the weaker cultivars of McKenzie and Harvest at both the 0% NaCl and 2% NaCl levels. Findings from the thermogravimetric analysis, with subsequent curve fitting, indicated a movement of water's association within the dough from being present as free water to being associated with the starch-fraction with the addition of 2% NaCl. Based on these findings it was postulated that the gluten network is not the only factor governing dough stickiness in reduced sodium formulations. Instead, the relationship between free water and that associated with the starch-fraction may also be another key factor governing dough stickiness. Based on the results two cultivars were chosen to move forward with to analyze the effect of alternative salts from the lyotropic series; one cultivar that had the best dough handling properties and low stickiness in reduced NaCl formulations (Pembina) and one cultivar showing the worst dough handling properties and high stickiness (Harvest).

In the third stage of this research (Chapter 5) the effect of salts from the lyotropic series ( $\text{NH}_4\text{Cl}$ ,  $\text{KCl}$ ,  $\text{NaCl}$ ,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{MgSO}_4$ ) on the handling properties of doughs prepared from a strong wheat flour cultivar (Pembina) and a weak wheat flour cultivar (Harvest) were investigated. Overall, Pembina developed a stronger gluten network than Harvest with the addition of all salt-types as indicated by the rheological results. Harvest's dough rheology appeared to be more sensitive to salt-type as indicated by the variability in the strength of the gluten network given the salt-type used. In all cases, Pembina had a lower dough stickiness than Harvest with each salt-type, however the extent of the change on dough stickiness was influenced by the different salt-types. Findings from the thermogravimetric analysis, and curve fitting, appear to indicate a difference in water's movement with the use of alternative salts dependent on the specific cultivar. Alternative salts for the cultivar Pembina had an effect of



water movement between free and gluten-fraction whereas alternative salts for Harvest had an effect of water movement between free and starch-fraction. This further points to the hypothesis that the gluten network is not the only governing factor in dough stickiness, but instead the strength of the gluten network masks water's change in association with the starch. This hypothesis would explain why Pembina exhibits lower stickiness at reduced sodium levels than Harvest which has a weaker gluten network formed. Based on the results  $\text{NH}_4\text{Cl}$  appears to improve dough handling properties and decrease stickiness in both flour cultivars and maintain the gluten network formation as seen in CLSM micrographs. Based on the results and literature it is concluded that  $\text{NH}_4\text{Cl}$  results in an increase in hydrophobic and hydrogen bonding between the glutenin polymers because of the competition of  $\text{NH}_4\text{Cl}$  with water. Therefore, there is a greater promotion of water-ion interactions over protein-water interactions resulting in greater protein-protein interactions capable to form the gluten network.

From this research it was concluded that the gluten network was not the only factor governing dough stickiness, where the starch-fraction was thought to also play an important role. The formation of a strong gluten network is thought to mask the presence of dough stickiness at reduced NaCl levels. It is hypothesized that in low NaCl dough formulations if the amount of water associated or absorbed by the starch-fraction could be increased, the dough stickiness problem in low sodium environments could be reduced. The reasoning behind this is because movement of water from the starch-fraction to the free water fraction with a decrease in NaCl was observed. Therefore if this movement of water can be prevented dough stickiness could be reduced. An increase in the amount of damaged starch is a potential way to increase the starch-fraction water absorption at low sodium levels. However further investigation into water mobility (using FTIR or NMR) in a range of NaCl levels is required before concrete conclusions can be made. There must also be an investigation into the impact of increasing damaged starch on the strength of the gluten network/dough given the present research indicates an inter-relationship of gluten network formation/strength and water mobility in the presence of dough stickiness. Ammonium chloride represents a promising alternative salt to NaCl in the prevention of dough stickiness. With that said, further studies on the effect of  $\text{NH}_4\text{Cl}$  on final bread quality needs to be investigated as well as the health implications of increased intake of  $\text{NH}_4\text{Cl}$  prior to use in bread formulations.

## **8. FUTURE STUDIES**

Dough is a complex multi-phasic system, and the handling properties become drastically altered as sodium levels are reduced. Although the focus of this research was the gluten proteins, a further understanding of the influence of the starch-fraction, levels of starch damage and non-starch polysaccharides on dough stickiness is of importance for understanding the dough stickiness phenomena. Having a complete understanding will enable the baking industry to develop formulation strategies for mediating effects of reduced salt in relation to the wheat quality and cultivars present. The approach will help reduce costs associated with equipment cleaning, maintenance or purchasing overhauls.

In the future, a more in-depth look at the impact of starch composition and structure on dough handling, stickiness and water mobility should be explored. For instance, a further investigation of the ratio of amylose to amylopectin should be examined as each have different physiochemical properties which could impact the dough strength and stability (Ramachandran et al., 2016). Wheat flours containing mutant genotypes for starch can result in either increased amylose (amylostarch) or increased amylopectin content (waxy starch containing up to 99-100% amylopectin) (Goesaert et al., 2005; Ramachandran et al., 2016). Ramachandran and others (2016) found that wheat flours containing greater amounts of waxy starch (with lower amylose and higher amylopectin) resulted in increased farinograph water absorptions which they related to both the ability of amylopectin to trap and hold water better than amylose and the increased susceptibility of waxy wheat to starch damage. Yi and others (2009) found that with increasing contents of waxy wheat flour added into dough formulations that there was an increase in dough stickiness, a decrease in resistance to extension, and an increase in extensibility. Thus the presence of waxy starch has an effect on water absorption, dough stickiness and dough strength and therefore it may be possible that some of the flour cultivars in this study contain the mutation for different levels of waxy wheat starch to differing degrees. The presence of this mutation would impact the results of differing levels of dough strength and stickiness among the four flour

cultivars. The strongest wheat flour with the lowest stickiness being Pembina may not have the starch mutation for waxy starch whereas the much weaker and stickier flour cultivar of Harvest may have a greater presence of this mutation thus increasing its level of dough stickiness and decreasing its strength. The presence or lack of this starch mutation could help explain the findings in the second section of this study with the movement of water association from free water to starch associated water, with the inclusion of NaCl, in all cultivars except for Pembina.

A further investigation into the specific subunit composition of the HMW and LMW insoluble glutenins within the four flour cultivars would also be important to explore, in relation to its impact on dough handling and stickiness. It has been discussed in the literature that the presence of certain subunits impart a strengthening effect and better dough handling properties whereas the presence of others have a weakening effect (Butow et al., 2002; Suchy et al., 2003; Barak et al., 2013). Barak and others found that cultivars with HMW-GS 2\*, 5+10 and 7+9 created doughs with higher development times and stability, whilst cultivars with HMW-GS N, 2+12 and 20 created doughs with lower stability and higher dough weakening. Butow and others found that different HMW-GS were more sensitive to changes in salt with those cultivars containing the over expression of the Bx7 HMW-GS gene being the most sensitive and also creating over-strong doughs. They concluded that the over expression of the Bx7 gene could compensate for the reduction in salt levels and achieve optimal dough handling properties as it would require less salt to achieve the same dough strength. Based on the findings in the current study, Pembina was the least impacted by the sodium reduction with regards to dough stickiness and dough strength, which could point to Pembina containing the over expression of the Bx7 HMW-GS gene.

A further examination of the effect of sodium chloride reduction on dough handling properties and water mobility in cultivars containing differing levels of damaged starch and LMW-GS should be undertaken. For instance, examining the effect of increased levels of damaged starch in low sodium formulations may be beneficial, since in Chapter 4 it was found that the movement of water's association was from free to starch with the addition of NaCl. Therefore if that water movement could be mimicked in a low sodium formulation by increasing the damaged starch this may help mitigate the occurrence of dough stickiness. However, the impact on the gluten network formation and strength must also be examined with differing levels of damaged starch. Another area of examination could be looking at the effect of increasing

LMW-GS concentrations within the flour in low sodium formulations to examine the role that LMW-GS may play in low sodium formulations and the occurrence of dough stickiness. It was found by Suchy and others (2003) that cultivars exhibiting similar HMW-GS subunit compositions had significant differences in dough quality properties and stated that differences in dough properties may be due to differences in LMW-GS and gliadin composition. D'Ovidio and Masci (2004) and Bonafede et al. (2015) discuss how the optimal HMW-GS alleles are becoming fixed in wheat breeding, leading researchers to investigate the contributions of both the LMW-GS and gliadins to certain cultivars' breadmaking quality. Bonafede and others (2015) found that certain LMW-GS coded for on the *Glu-3* loci (i.e., *Glu-A3f*, *Glu-B3b*, *Glu-B3g* and *Glu-B3i<sub>Man</sub>*) were associated with the highest values when examining gluten strength parameters whereas other LMW-GS (i.e., *Glu-A3e*, *GluB3a*, and *Glu-B3i<sub>Chu</sub>*) were associated with low quality and weak gluten. This may be a useful avenue of insight given the findings in the gluten fractionation of Pembina having the greatest amount LMW-GS and having the best dough handling properties and lowest stickiness in the low sodium formulations. In the examination of dough stickiness, based on the reproducibility of the Chen and Hoseney (1995a) method within this study and comparing it to other studies using the same method, a further examination into dough stickiness testing techniques should be undertaken.

The knowledge gained in Chapter 5 on the effect of alternative salts compared to sodium chloride on the dough rheology, morphology, stickiness, and water mobility could be expanded by changing the method of salt addition to the formulation. For instance, within the current study the salts were added on a flour weight basis as this is what would be done in industry. However, the alternative salts have different ion sizes and thus may have different hygroscopic capacities and therefore could have different effects on water's association given their ion concentrations. Within the research by Butow and others (2002) they utilized an equimolar cation concentration as they stated that ions differ in their ability to bind water and thus may alter the hydration of each flour component. Therefore further analysis could be done using similar ion concentrations (the same molarities) for each of the salt-types within the dough formulations to remove the factor of different ion concentrations being the explanation for the differences observed in the dough rheology, morphology, stickiness and water association within the different flour cultivars. Ratios of substitution with sodium chloride in the total molarities could also be tested by using the alternative salts which gave similar properties to sodium chloride. For instance,

Salovaara (1982a) investigated the substitution of NaCl at 20 and 40% replacement levels with alternative salts based on sodium ion equivalents, whereas Kaur and others (2011) appear to have done a substitution of 25 and 50% on a flour weight basis in the similar manner they added NaCl, however it is unclear.

Furthermore, an examination should be done on how the changes in salt levels, salt-types, and flour cultivars translate into final loaf quality and shelf life. Therefore baking trials would be an important aspect to the analysis to ensure that changes translate into a high quality final product. For instance, within the thermogravimetric analysis in Chapter 5 with the alternative salts ( $\text{NH}_4\text{Cl}$ , NaCl, and  $\text{MgCl}_2$ ), after the application of the final  $200^\circ\text{C}$  (which is similar to the temperature that a loaf of bread would be baked at) the colour of the little dough balls were different for each salt-type. The ammonium chloride resulted in a baked colour of dark brown, similar to that of pumpernickel bread, sodium chloride resulted in a golden brown colour and magnesium chloride resulted in a slightly darker caramel colour. Butow and others (2002) investigated the use of alternative salts and different cultivars but did not do baking tests and stated that studies done by Holmes and Hoseney (1987) and He and others (1992) found that although certain salts increased dough strength, they did not improve loaf volume. Therefore it would be interesting to do baking trials with the alternative salts to examine the effect on loaf colour, volume, crumb texture and crumb grain. This addition of baking trials would bring the analysis of preventing dough stickiness within low sodium formulations full circle to the effect on the most important aspect, the final bread product for the consumer.

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